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Fully replicable and automated retention measurement setup for characterization of bio-adhesion

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ABSTRACT

The retention model by Rao and Buri is often used to characterize microparticles and other drug delivery systems for their bio-adhesive properties. Currently, these experiments are performed on customized setups, reducing reproducibility of results obtained in different labs. As a solution, we propose a fully replicable retention model, which can be constructed by parts mostly made by 3D printing and laser cutting as well as a limited amount of other easy to source and commercially available parts. In addition of being fully replicable, the setup features integration of a climate-controlled chamber, a peristaltic pump and an autosampler, thereby enabling fully automated but customized control of the experiments. Using the presented retention model setup and an automated experimental sequence, the setup has been proven capable of investigating mucoadhesion of differently shaped particles to porcine intestinal tissue.

Specifications table

Hardware name	*Integrated retention model setup*
Subject area	• Medical (e.g. Pharmaceutical Science)
Hardware type	• *Other [Evaluation of bio-adhesive properties of* e.g. *drug delivery devices, drug formulations, adhesive structures]*
Open Source License	CC BY 4.0, CC BY-NC-SA 4.0 and MIT license
Cost of Hardware	*400–500 USD*
Source File Repository	https://doi.org/10.17632/v2rdbwpx7k.1

1. Hardware in context

The retention model, also referred to as ex-vivo flow model or flow-through method, was first introduced by Rao and Buri in 1989 and was developed as a method to assay the bio-adhesion of polymers and microparticles to rat gastro-intestinal tissue [1]. Since then it has proven to be a very versatile method, which is commonly used to investigate the mucoadhesive

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properties of various drug delivery formulations and therefore is regarded to be one of the main methods to measure mucoadhesion [2]. In this regard, the method has been applied to determine the mucoadhesiveness of e.g. thiomer microparticles to porcine intestinal tissue, metformin hydrochloride/chitosan microparticles to porcine buccal mucosal tissue or microfabricated janus devices to porcine intestinal tissue [3–5]. The precise control of experimental conditions, such as temperature, humidity, content of simulated biological fluids as well as the flow rate is considered to be very important as the lack of it can negatively affect reproducibility of the experiments [2].

The simplicity and versatility of the core elements of the experimental setup in general, a pump connected through a tube to a tissue holder, which holds a biological sample tissue at a specific angle, motivates researchers to construct their own customized setup. On the one hand, the construction of home-made setups provides a lot of flexibility and design freedom to researchers, but on the other hand it leads to a lack of reproducibility as well as comparability in the scientific community as there is no common standard with regards to the way setups are built and the experiments are performed. Furthermore, information about how these setups are constructed and/or used is often missing so that a replication of the same setup used for a published work is not possible. The commonly used experimental setups also exhibit various degrees of complexity. In contrast to the simplest system consisting of a pump, a tubing and a tissue holder, the system can get more sophisticated when temperature and humidity control of the ambient climate are included. Consequently, reproducing such a system can become more difficult.

The emergence of affordable 3D printing and other rapid prototyping techniques (e.g. laser cutting) has triggered the open sharing of design files for customized lab equipment, also called open labware [6,7]. The principle of open labware is that, by freely accessing the design files, it can be for example 3D printed and therefore easily replicated everywhere where there is a 3D printer available (e.g. in Universities or public maker spaces). Based on this approach, different types of lab equipment with different degrees of complexity have been developed and instructions for their replication are available in the literature. Among those are standard laboratory equipment and appliances, such as a sample rotator mixer/shaker and optical fixtures, but also rather advanced laboratory tools such as a microsyringe autosampler and a Raspberry Pi/Arduino-based fluorescence microscope with modular features for e.g. optogenetic analysis [8–11].Through the use of 3D printable components and widely available low-cost accessories, customized open labware can drastically decrease cost and at the same time yield a very high return on investment for the global scientific community [6,12].

In this paper, we propose and share designs for an open labware-based retention model setup that aims at balancing reproducibility with customizability and flexibility by being modular and upgradable. The setup can be fully and easily replicated by the use of 3D printed and laser-cut parts as well as commonly available commercial components. As a free and open available development platform, the setup could in the future offer a standard for retention model experiments that researchers could refer to.

2. Hardware description

The retention model setup (Fig. 1) was designed to fulfill certain requirements. Above all, it was considered important that 3D printing and laser cutting can be used to fabricate most of the parts, therefore making the replication process as simple as possible. All other parts that are needed should be cheap and easy to source. For this reason and to integrate several required functionalities in one control loop, we chose the Arduino Mega 2560 (microcontroller)/ RAMPS 1.4 (Arduino shield) combination, which is frequently used for the control of RepRap 3D printers. The RAMPS 1.4 shield possesses all required circuits and connectors to use it in combination with the Arduino for the control of temperature, humidity, pump flow and autosampler rotation. The simple control of temperature and humidity is accomplished with the use of relays, a ceramic infrared heat lamp, an ultrasonic mist fogger and three fans and two DHT22 temperature and humidity sensors. The basic design of the system is kept in a modular way by arranging all components on a breadboard-style base plate, therefore allowing further customization and upgradability. The integration of a peristaltic pump as well as a rotary autosampler adds versatility and precision to the setup as it enables the execution of customized and fully automated program sequences.

In summary, the presented retention model setup can offer:

- **Improved repeatability of flow retention experiments** (e.g. through climate-controlled environment and automation)
- **Improved reproducibility of flow retention experiments** (when different researchers use the same setup)
- **Expanded functional range** (by integration of peristaltic pump, auto-sampler and automation with Arduino microcontroller e.g. fully automated customized programs for experiments)

3. Design files

3.1. Design files summary

All design files listed in this section are available for download from the Mendeley data repository. Most of the components were designed specifically for this project using SolidWorks 2015 (Dassault Systèmes SolidWorks Corporation, USA) software, however in some cases resources from other projects were used to obtain needed components. An online tool was used to generate the climate chamber box design with outside dimensions of $291 \times 296.5 \times 400$ mm, a material

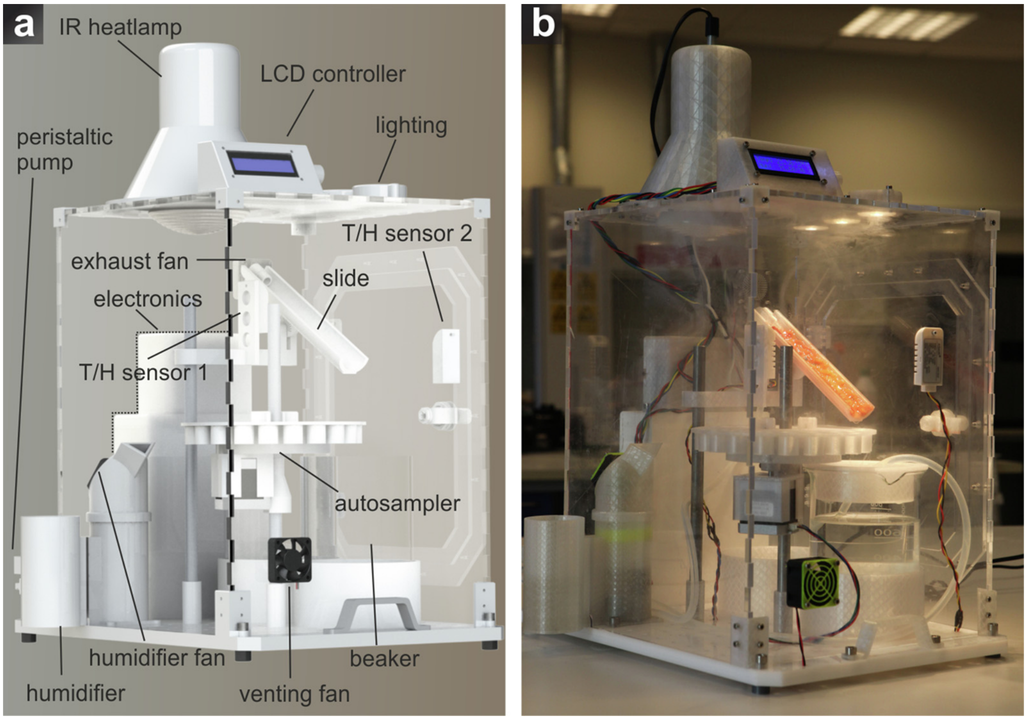

Fig. 1. 3D rendering of technical drawing (a) and photo (b) of completed setup during use.

thickness of 5 mm, finger slots with a tab length of 25 mm and a laser-cut kerf of 0.1 mm, which then was modified according to the need of this project [13]. *lock_new-lever.SLDPRT* was designed as a modification to an online available lock design to fit the specifications given by the climate chamber design presented in this work [14]. *parametric_butt_hinge_3.5.2.scad* OpenSCAD library was used to generate a hinge design [15]. *Getriebe.scad* OpenSCAD library was used to generate gear designs needed for the autosampler [16]. *pump_housing.SLDPRT* was designed as a modification to an open source precise peristaltic pump [17]. *humidifier_2.0.SLDPRT* and *humidifier_fan.SLDPRT* are a modified redesign of a desktop humidifier [18].

Design file name	Designator	File type	Open source license	Location of the file
slide.SLDPRT	slide	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-781defc0-a3f3-46b6-8ce8-5ced1d95cc1b
height_adjustable_slide_holder.SLDPRT	slide	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-563eee42-0e45-499d-a46e-a088561c22c5
baseplate1.SLDPRT	baseplate + holders	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-80329d12-4c87-4455-a5c0-4024d06b0d07
baseplate2.SLDPRT	baseplate + holders	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-9477ee84-5241-4143-8207-88961c00c4cd
beaker_fix.SLDPRT	baseplate + holders	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-ce618dff-541f-4b43-878d-c55ed9c7f692
rod_holder.SLDPRT	baseplate + holders	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-fca70f17-11d4-4000-8aac-8f8195ae5839
handle.SLDPRT	baseplate + holders	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-31887229-cec2-40cd-8f8f-04c3da6e9eac
foot.SLDPRT	baseplate + holders	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-35cb961a-5acb-48da-808a-305b867fedd3
outside_corner.SLDPRT	baseplate + holders	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-21936225-0cf4-4336-b61f-fdeebc79175a

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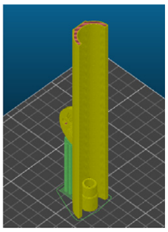
Design file name	Designator	File type	Open source license	Location of the file
outside_corner_mirrored.SLDPRT	baseplate + holders	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-5ddd61a8-4296-4725-88f6-9669ee3a7d90
outside_corner_regular.SLDPRT	baseplate + holders	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-4e48b474-d19b-409d-8bdb-3d0cf36e35c6
outside_corner_regular_mirrored.SLDPRT	baseplate + holders	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-10681b40-afaa-4439-8cff-a7f9a70a2261
box_back.SLDPRT	climate_chamber	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-3a3e553b-ade5-4e77-9f01-e37d4be0f26f
box_front.SLDPRT	climate_chamber	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-32e5aa6b-0cbd-4f88-8bc5-376b115a2e0e
box_top.SLDPRT	climate_chamber	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-551f2c96-32e0-4373-8028-93dc933ee9b4
box_side.SLDPRT	climate_chamber	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-b144cb72-7a03-4efd-b5c6-46075236d17c
box_doorside.SLDPRT	climate_chamber	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-13ad80b0-dd46-4a99-8c28-35c6951de02b
box_doorframe.SLDPRT	climate_chamber	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-62a0c042-c043-4cdc-8e24-7b0ffcf52ff8
box_door.SLDPRT	climate_chamber	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-6cb90d39-8f9f-4f7f-8b7e-24d1d3cfd4b8
corner_top.SLDPRT	climate_chamber	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-548386ac-2d2b-427e-b2b3-78a31cf5556e
lock_new-lever.SLDPRT	climate_chamber	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-0a815ab9-ed71-42a3-9a88-383ce179c1fc
hinges.scad	climate_chamber	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-067d6e80-2137-4131-b1e5-b5c9d801aaf4
parametric_butt_hinge_3.5.2.scad	climate_chamber	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-db425313-505d-4aac-a8e6-0d08a95a721f
humidifier_2.0.SLDPRT	humidifier	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-bd52111e-0cf2-4e71-aed2-1672d54e5dda
humidifier_fan.SLDPRT	humidifier	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-8cf99966-8a8f-45a8-bcd0-b3805e225d71
humidifier_mesh.SLDPRT	humidifier	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-1a3ffecf-3e66-4bc4-8965-f77e8719a4a8
pump_housing.SLDPRT	peristaltic_pump	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-f8859efc-3167-4de4-9330-5f0536d6f100
lamp_case.SLDPRT	electronics	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-41fe311c-9e60-462f-8c56-dc6cf125ce41
controller_housing.SLDPRT	electronics	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-90e34518-094c-4f20-b8a6-8138fb29aead
controller_knob.SLDPRT	electronics	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-8807e60f-3ca8-4b64-80eb-d9cc173d2628
plastic_washer.SLDPRT	Electronics	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-2c2ba8e9-4670-4c34-81cf-d0f3e24a1f6d
electronics_housing.SLDPRT	electronics	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-a36711b9-c1f7-42d0-bcde-dbc55895c5ef
ikea_led_housing.SLDPRT	electronics	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-79d684b4-3922-401a-a39f-c92f0132197a
ssr_housing.SLDPRT	electronics	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-aa0f6697-9346-44bc-9f3f-f7e466360586
warning_sign.SLDPRT	electronics	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-b789ad59-0be5-4337-bada-4cf76a579aa9
autosampler_gear_drive.scad	autosampler	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-170515c6-3f7b-4ee5-8a6e-45587ef86658

(*continued*)

Design file name	Designator	File type	Open source license	Location of the file
Getriebe.scad	autosampler	CAD	CC BY-NC-SA 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-925f4490-e5b8-4eb0-8008-ec3a7d06abc6
gear_drive_cover.SLDPRT	autosampler	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-90f603ad-bcdf-4933-bbd4-e3ead0536def
geardrive_motormount.SLDPRT	autosampler	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-f80c4f4e-0051-4daa-a327-99926f31bed9
thumbnut.SLDPRT	autosampler	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-8e64770b-31c4-4534-8aaa-6ac58dca8e7d
eppiholder.SLDPRT	autosampler	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-a5f1f500-9b1a-4a49-b896-ea72262da8c1
eppiholder2.SLDPRT	autosampler	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-66bab9da-25e5-4c61-a10e-1a68829d288b
15ml_holder.SLDPRT	autosampler	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-546f8d2a-26e7-458c-b054-beb8ae2550fd
50ml_holder.SLDPRT	autosampler	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-f7ee7661-d6e0-492b-82af-7a2e088a40aa
mesh_holder.SLDPRT	autosampler	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-f039ea8c-6adb-43f3-ad91-c91d3920c2ee
small_mesh.SLDPRT	autosampler	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-6845677b-c896-4cae-a08a-5de65beaf41c
mesh.SLDPRT	accessories	CAD	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-69ece420-05b5-4f9c-8277-2f609795bb58

3.2. 3D printing files

All 3D printing files are available in the STL file format for download from the linked Mendeley data repository. The table gives an overview of all files that have to be 3D printed in order to complete the project. Furthermore, the table gives information about how many replicates of the components are required, how they look and in which orientation they should be 3D printed (images were generated with 3D printing slicing software). For this project, usually all parts were 3D printed with a 0.4 mm nozzle, a layer height of 0.2 mm and 20% infill from PETG filament (2 spools of 1 kg each are sufficient). In some cases, the use of support material was necessary. The support material can be seen in the images as stacks of green lines. As the object humidifier_2.0 is supposed to contain water during the application, it should be printed with a higher infill density and increased amount of shells. In case water would still be leaking from the object, the authors recommend sealing the reservoir by impregnation with silicone or epoxy resin. *lock_hole-screw.stl*, *lock_big-nut.stl*, *lock_key.stl* and *lock_small-nut.stl* were obtained from an open lock design and renamed [14]. *pump_case_bottom.stl*, *pump_case_top_120.stl*, *bearing_mount_top.stl* and *bearing_mount_bottom_01.stl* were obtained from an open source peristaltic pump design [17]. *40mm_Fan_grill_final.stl* was likewise obtained from external source as a publicly distributed design [19]. All other STL files were exported from design files listed in 3.1. *thumbnut.stl* requires the insertion of an M3 nut during the 3D printing procedure.

Design file name	Designator	No. of required prints	Image	Open source license	Location of the file
slide.stl	slide	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-c9a6f9ff-2a35-4284-99c7-57167272eed8

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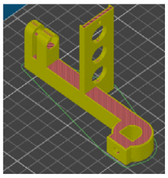
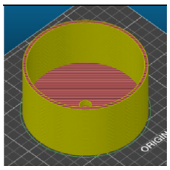
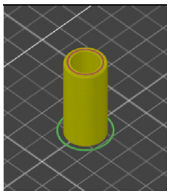
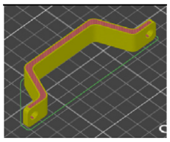
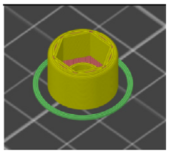
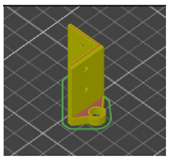
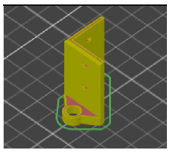
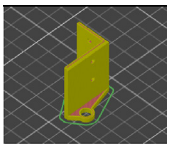
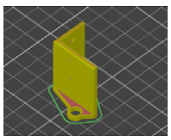
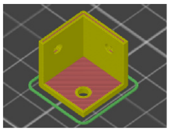
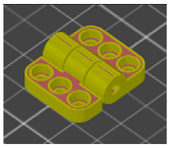
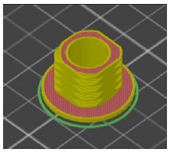
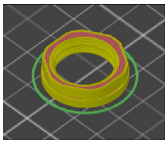
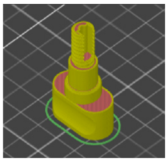
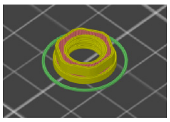
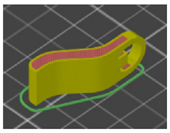
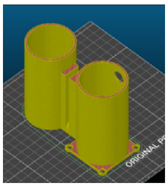
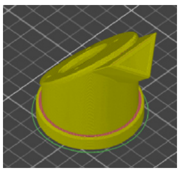
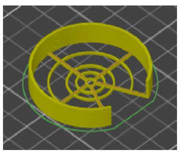
Design file name	Designator	No. of required prints	Image	Open source license	Location of the file
height_adjustable_slide_holder.stl	slide	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-61eb404c-4006-4bcc-93e1-b5dcd900f8e7
beaker_fix.stl	Baseplate + holders	1 (+1 optional)		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-70c8968f-f077-4d1b-a71f-961ebe3b075d
rod_holder.stl	Baseplate + holders	2		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-4eb3f47c-37b5-4d52-9990-58a8f5ab07ab
handle.stl	Baseplate + holders	2		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-a8f11a00-b7ae-4f70-bc1c-a11b844ae334
foot.stl	Baseplate + holders	5		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-1d1ac0ca-1ce6-4bfc-869c-11a522e74fdc
outside_corner.stl	Baseplate + holders	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-cc7009c3-7611-425c-8719-f94f032eaa7f
outside_corner_mirrored.stl	Baseplate + holders	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-4b890b16-5a86-4621-b0b7-a130139f2f50
outside_corner_regular.stl	Baseplate + holders	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-aa5c8c37-fccf-45c7-b163-296f5c90adda
outside_corner_regular_mirrored.stl	Baseplate + holders	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-16f5ebe5-7d85-4a43-ab45-eaf5d4c8323a

(*continued*)

Design file name	Designator	No. of required prints	Image	Open source license	Location of the file
corner_top.stl	climate_chamber	4		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-f8cda31e-36ba-4f31-8000-5309c9cd53eb
hinge.stl	climate_chamber	2		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-1dac2836-6f7b-4ca6-8db3-8200d69cfdf0
lock_hole-screw.stl	climate_chamber	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-fc6376b2-bec9-4570-a670-fcbf735b2fec
lock_big-nut.stl	climate_chamber	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-65ddfb3a-19a7-4d23-8239-fe902607e966
lock_key.stl	climate_chamber	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-84521c78-0051-4c60-a3f6-83b23fca4e07
lock_small-nut.stl	climate_chamber	2		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-4cec0da0-dfff-4840-b6c7-1dcaae9f02bb
lock_new-lever.stl	climate_chamber	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-9295a859-703b-4ed6-8d1c-8d58632e335b
humidifier_2.0.stl	humidifier	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-0ecc598a-f507-4e6c-950e-d075a19771d1
humidifier_fan.stl	humidifier	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-d499e6e3-5094-4cb2-90db-e1b1b15b1839
humidifier_mesh.stl	humidifier	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-e43beba0-5d73-484b-85b5-0740e147ba22

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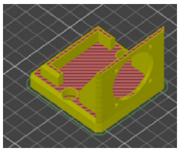
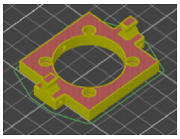
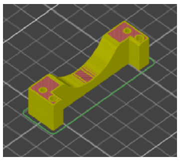
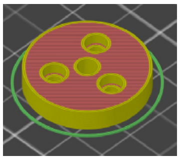
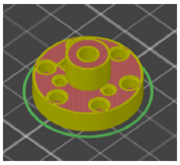
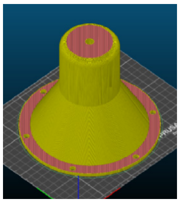
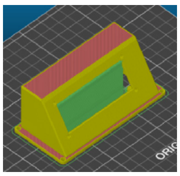
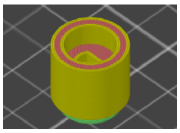
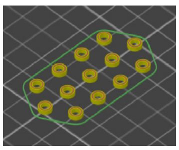
Design file name	Designator	No. of required prints	Image	Open source license	Location of the file
pump_housing.stl	peristaltic_pump	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-f3ad393e-be08-4d00-a759-77090dea53c5
pump_case_bottom.stl	peristaltic_pump	1		CC BY-NC-SA 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-c4245fd7-e3db-4622-8c43-be69fa121f01
pump_case_top_120.stl	peristaltic_pump	1		CC BY-NC-SA 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-5d66fb55-5545-42b7-adaf-e2627e553c6a
bearing_mount_top.stl	peristaltic_pump	1		CC BY-NC-SA 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-6495ae60-a59b-4b8d-b124-4f822789ec8a
bearing_mount_bottom_01.stl	peristaltic_pump	1		CC BY-NC-SA 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-03037277-4dc0-415f-81d1-45771e61c192
lamp_case.stl	electronics	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-736f9b78-cd19-451a-a49a-05cc5f1980cf
controller_housing.stl	electronics	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-ef349445-12c3-4175-9fa0-710c892cedd7
controller_knob.stl	electronics	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-1c7d08be-ebcd-46bf-8be8-e1dfe9c1f6fa
plastic_washer.stl	electronics	13		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-11901593-e905-4aec-bbdd-31e6cd6d0cbf

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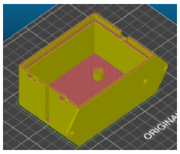
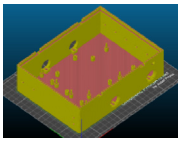
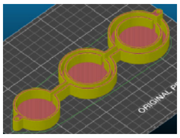
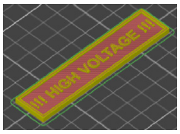
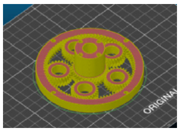
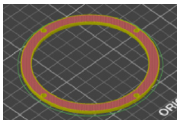
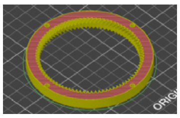
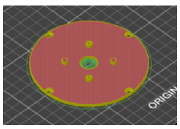
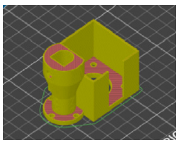
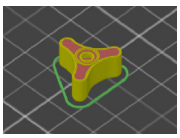
Design file name	Designator	No. of required prints	Image	Open source license	Location of the file
ssr_housing.stl	electronics	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-359171ad-3f0d-4256-87da-0525db84cf71
electronics_housing.stl	electronics	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-eb649bab-4b70-4656-83a0-61fe4ca4d5b0
ikea_led_housing.stl	electronics	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-4c41c6e1-68c8-408b-ad76-dcbe8062e9f8
warning_sign.stl	electronics	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-8aed6cff-6742-47ac-a2c1-21aff6c50473
planetary_gear_drive.stl	autosampler	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-3e9e847d-6401-4c71-85c6-a1450d9f7814
planetary_gear_drive_shim.stl	autosampler	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-5d50bd03-7413-4068-8c97-d40e43208432
ring_gear.stl	autosampler	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-c4591cdc-939d-4458-af48-453617c09f78
herringbone_gear.stl	autosampler	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-155822db-d3e7-42e8-bef5-2c62454a2de0
gear_drive_cover.stl	autosampler	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-7b4f305a-c6be-462e-b525-b7d24dabf314
geardrive_motormount.stl	autosampler	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-8bc6d707-c249-4d11-8100-863826478400
thumbnut.stl	autosampler	2		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-8738655c-f8ef-4233-9595-d73798a5713c

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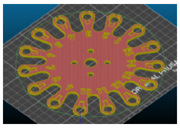
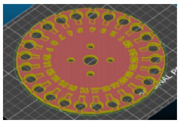
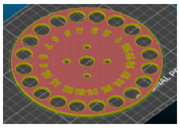
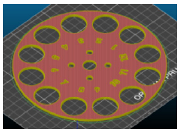
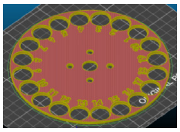
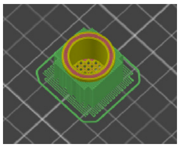
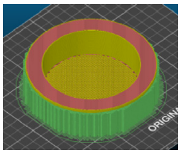
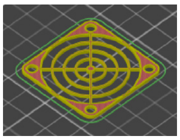
Design file name	Designator	No. of required prints	Image	Open source license	Location of the file
eppiholder.stl	autosampler	optional		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-205db04e-f360-490e-ae67-e3be76948c91
eppiholder2.stl	autosampler	optional		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-47bdbad3-ce06-43bf-b2e7-697cc0b897e9
15ml_holder.stl	autosampler	optional		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-b731ed67-8911-488d-a229-eb75bf43dd43
50ml_holder.stl	autosampler	optional		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-36fb7641-4e9b-49cd-81f6-a510c9be3f4b
mesh_holder.stl	autosampler	optional		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-0617f2b1-4cfb-49a6-9f3f-6c720510fa70
small_mesh.stl	autosampler	optional		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-8bce4dc8-c8c0-4eea-834a-94e492bff9a4
mesh.stl	accessories	1		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-00ef4678-21ca-44d7-96a3-0a69202ce981
40mm_Fan_grill_final.stl	accessories	5		CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-350630e4-553a-4fab-aaf5-18ec21fd3b18

3.3. Laser cutting files

To complete the construction of the presented retention model setup, it is necessary to cut out several designs from sheets of polymer. All designs are available as DXF files for download from the Mendeley data repository. The DXF files were created using SolidWorks 2015 (Dassault Systèmes SolidWorks Corporation, USA) and CorelDRAW X7 (Corel Corporation, Canada) software. In this work, all designs were designed to fit in the A3 paper format and were cut from acrylic using a CO_2 laser cutter (Epilog Mini 18). The designs *baseplate1_labeled.dxf* and *baseplate2.dxf* were cut from 6 mm acrylic, while all other designs except for *woodplate_ssr.dxf* were cut from 5 mm acrylic. *woodplate_ssr.dxf* was cut from 3 mm high density fiber board.

Due to the limited chemical resistance of acrylic, the authors recommend to not use solvents (e.g. ethanol) for cleaning of these boards. All sheets can also be cut from different materials and also by using different cutting methods (such as CNC routing).

Design file name	Designator	File type	Open source license	Location of the file
baseplate1_labeled.dxf	baseplate + holders	DXF	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-9a09fa99-1774-477d-bd26-f996bc76a0b6
baseplate2.dxf	baseplate + holders	DXF	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-588c68a9-65c1-469f-b1d8-d62301eb4d21
box_back.dxf	climate_chamber	DXF	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-b47d6038-864a-4795-8cc1-fa53eda771f8
box_doorside + electronics_cover.dxf	climate_chamber and electronics	DXF	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-350762fc-7672-4456-a793-bedb8b110ea8
box_front.dxf	climate_chamber	DXF	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-cd503df4-d2fe-4398-baf6-adf9036fcbea
box_side.dxf	climate_chamber	DXF	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-7cf94960-4774-4be6-b282-b3b24a88676f
box_top + SSR_cover.dxf	climate_chamber and electronics	DXF	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-2b16de05-237c-4063-93f2-8b3c78988c5c
door + frame.dxf	climate_chamber	DXF	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-fc0759f6-815c-4884-8200-4657142a6404
woodplate_ssr.dxf	electronics	DXF	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-fc0759f6-815c-4884-8200-4657142a6404

3.4. Software

An Arduino Mega 2560 microcontroller is used to control the feedback loop for the climate control as well as to control an autosampler and a peristaltic pump in combination with an Arduino Pro Mini. A rotary encoder and LCD display serve as a feedback and input interface to control the operation of the microcontrollers. The used Arduino sketches are available for download as ino files from the Mendeley data repository. In order to integrate the function of the DHT22 temperature and humidity sensor as well as an I2C LCD display, external libraries were employed. *Adafruit_Unified_Sensor* and *DHT-sensor-library-master* were obtained from Adafruit and *Newliquidcrystal_1.3.5* was obtained from an open source [20–22].

File name	File type	Open source license	Location of the file
main.ino	Arduino	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-ab7e2152-f518-477c-b5ae-5d2d7802ca5a
mini_pump_control.ino	Arduino	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#file-c3a6e562-26e7-437f-b0cc-5518eb218009
Adafruit_Unified_Sensor	Arduino library	MIT license	https://doi.org/10.17632/v2rdbwpx7k.1#folder-4195312f-f10a-4ee4-af9e-67c4293b0bd6
DHT-sensor-library-master	Arduino library	MIT license	https://doi.org/10.17632/v2rdbwpx7k.1#folder-73fe49c3-b8e2-46a6-b2bf-14cea79fbf67
Newliquidcrystal_1.3.5	Arduino library	CC BY 4.0	https://doi.org/10.17632/v2rdbwpx7k.1#folder-e15da967-8b32-4102-8fc8-1a21e3f571fd

4. Bill of materials

4.1. Bill of materials

In addition to the 3D printed and laser-cut components of the system, some parts must be obtained from external sources. The table gives an overview of which and how many items need to be purchased. While all listed components should be easy to source, the pricing of those can vary a lot. The displayed costs in the table are calculated based on the parts we have obtained or on the prices we could find at the given sources at time of publication.

Designator	Component	Number	Cost per unit - currency [USD]	Total cost -Currency [USD]	Source of materials	Material type
Baseplate + holders	6 mm acrylic sheet (297 × 420 mm)	2	15.00	30.00	Hardware store	Polymer
baseplate + holders	5 mm acrylic sheet (297 × 420 mm)	6	13.00	78.00	Hardware store	Polymer
baseplate + holders	12 mm aluminum rod (300 mm)	2	1.60	3.20	Hardware store	Metal
humidifier	24 VDC Ultrasonic mist fogger	1	11.38	11.38	Amazon	Electronics
humidifier	12 VDC 40 × 10 mm fan	1	5.00	5.00	Mouser	Electronics
climate_chamber	12 VDC 40 × 20 mm fan	2	5.00	10.00	Mouser	Electronics
electronics	12 VDC 40 × 20 mm fan	1	5.00	5.00	Mouser	Electronics
electronics	150 W Ceramic infrared heat lamp for reptiles	1	28.50	28.50	Amazon	Electronics
electronics	Aluminum tape	1	7.30	7.30	RS Components	Tape
electronics	Ceramic heat lamp power socket	1	8.50	8.50	Amazon	Electronics
electronics	Kudom 10 A 280 VAC Solid State Relay Panel Mount	1	19.20	19.20	RS Components	Electronics
electronics	inline fuse holder	1	2.36	2.36	RS Components	Electronics
electronics	3A cartridge fuse	1	0.27	0.27	RS Components	Electronics
electronics	Arduino Mega 2560	1	9.00	9.00	Ebay	Electronics
electronics	RAMPS 1.4 Arduino Mega Shield	1	9.00	9.00	Ebay	Electronics
electronics	A4988 Stepper drivers	2	1.80	3.60	Ebay	Electronics
electronics	Arduino Pro mini	1	5.13	5.13	Ebay	Electronics
electronics	USB to serial converter for Arduino	1	2.50	2.50	Ebay	Electronics
electronics	Rotary Encoder	1	2.80	2.80	Ebay	Electronics
electronics	Ikea Ledberg spots (pack with 4)	1	13.00	13.00	Ikea	Electronics
electronics	DF Robot Gravity Digital 5A Relay Module	2	4.90	9.80	Mouser	Electronics
electronics	I2C 2 × 16 LCD	1	6.00	6.00	Ebay	Electronics
electronics	DHT22 Sensor	2	15.00	30.00	Mouser	Electronics
electronics	5A 250 VAC Toggle switch	1	2.85	2.85	RS Components	Electronics
electronics	LM2577 LED DC/DC boost converter	1	5.70	5.70	Ebay	Electronics
electronics	12 V DC 7A LED power supply	1	11.40	11.40	Ebay	Electronics
electronics	colored wires 0.14 mm2 (+fem. pin headers)	1	10.00	10.00	Amazon	Electronics
autosampler	Nema 17 stepper motor	1	14.00	14.00	Ebay	Electronics
peristaltic_pump	Nema 17 stepper motor	1	14.00	14.00	Ebay	Electronics
peristaltic_pump	Needle bearing HK 0408	3	4.30	12.90	RS Components	Metal
peristaltic_pump	4 × 14 mm pin	3	5.70	17.10	Amazon	Metal
peristaltic_pump	ID 4 mm 1.6 mm wall thickness silicone tubing (1 m)	1	6.61	6.61	Lab supplier	Polymer

(*continued*)

Designator	Component	Number	Cost per unit - currency [USD]	Total cost -Currency [USD]	Source of materials	Material type
General	PETG filament (2000 g)	1	45.68	45.68	3D print supplier	Polymer
General	M6 × 25 hex head cap screws	10	0.28	2.76	Amazon or hardware store	Metal
General	M6 × 20 hex head cap screws	10	0.25	2.50	Amazon or hardware store	Metal
General	M6 × 16 hex head cap screws	10	0.56	5.59	Amazon or hardware store	Metal
General	M6 × 12 hex head cap screws	10	0.22	2.21	Amazon or hardware store	Metal
General	M6 × 10 hex head cap screws	10	0.22	2.21	Amazon or hardware store	Metal
General	M6 Nut	30	0.18	5.34	Amazon or hardware store	Metal
General	M4 × 10 Button head screws	20	0.20	3.95	Amazon or hardware store	Metal
General	M4 × 8 hex head cap screw	5	0.20	1.00	Amazon or hardware store	Metal
General	M3 × 30 hex head cap screws	20	0.21	4.2	Amazon or hardware store	Metal
General	M3 × 25 hex head cap screws	10	0.18	1.84	Amazon or hardware store	Metal
General	M3 × 20 hex head cap screws	10	0.17	1.72	Amazon or hardware store	Metal
General	M3 × 16 hex head cap screws	10	0.17	1.68	Amazon or hardware store	Metal
General	M3 × 10 hex head cap screws	50	0.10	4.85	Amazon or hardware store	Metal
General	M3 × 8 hex head cap screws	20	0.20	4.00	Amazon or hardware store	Metal
General	M3 × 6 hex head cap screws	10	0.16	1.62	Amazon or hardware store	Metal
General	M3 × 25 hex head countersunk screw	5	0.27	1.35	Amazon or hardware store	Metal
General	M3 × 6 hex head countersunk screw	5	0.25	1.24	Amazon or hardware store	Metal

(*continued on next page*)

(*continued*)

Designator	Component	Number	Cost per unit - currency [USD]	Total cost -Currency [USD]	Source of materials	Material type
General	M3 × 10 grub screw	10	0.23	2.34	Amazon or hardware store	Metal
General	M3 × 8 grub screw	10	0.23	2.34	Amazon or hardware store	Metal
General	M3 Nut	10	0.17	1.70	Amazon or hardware store	Metal

4.2. Required tools

For the fabrication and assembly process, access to the following tools is required:

- 3D printer
- Laser cutter
- M3, M4 and M6 thread cutting tool
- Soldering equipment
- Cable stripper
- Pliers
- Crimping tool for pin headers
- Adjustable spanner
- Allen keys:
  - o 1.5 mm (M3 grub screws)
  - o 2 mm (M3 hex head countersunk screws)
  - o 2.5 mm (M3 hex head cap screws and M4 button head screws)
  - o 3 mm (M4 hex head cap screws)
  - o 5 mm (M6 hex head cap screws)

5. Build instructions

This section thoroughly describes the assembly of the presented retention model setup. As the initial assembly by the authors was carried out in an iterative approach with repeated interruptions for documentation purposes, a precise time estimate cannot be given at this point. However, based on initial experiences and under the circumstance that all required parts (including 3D printed and laser-cut parts) are available, an assembly duration of 2–3 h in total may be expected.

5.1. Assembly of baseplate

To begin the construction of the retention model setup, we recommend starting with the assembly of the baseplate. To make the assembly process easier, numberings are included in the file *baseplate1_labeled.dxf*, which can be engraved into the sheet using the raster engraving function of the laser cutter. The single steps of the procedure are laid out in Fig. 2.

1. Insert one M6 nut into the 5 feet (*foot.stl*) each (Fig. 2a).
2. Stack baseplate1 (*baseplate1_labeled.dxf*) onto baseplate2 (*baseplate2.dxf*). Insert two M6 × 20 hex head cap screws from the top into slots B and D of baseplate1 and fasten them to the M6 nuts in two feet at the bottom of baseplate 2 (Fig. 2b; green highlights).
3. Insert two M6 × 25 hex head cap screws into each of two of the climate chamber corners (*outside_corner.stl* and *outside_corner_mirrored.stl*) and then through baseplate1 in slots A and C (Fig. 2b; purple highlights). Then fasten the bolts to the M6 nuts placed in two more feet. Now, the two baseplates should be attached to one another in the four corners A, B, C and D.
4. Insert two M6 × 15 hex head cap screws into the two left outside corners (*outside_corner_regular.stl* and *outside_corner_regular_mirrored.stl*) and fasten them to two M6 nuts placed on the bottom side of slots 14 and 184, so that the corners are directed inwards (Fig. 2b; black highlights).

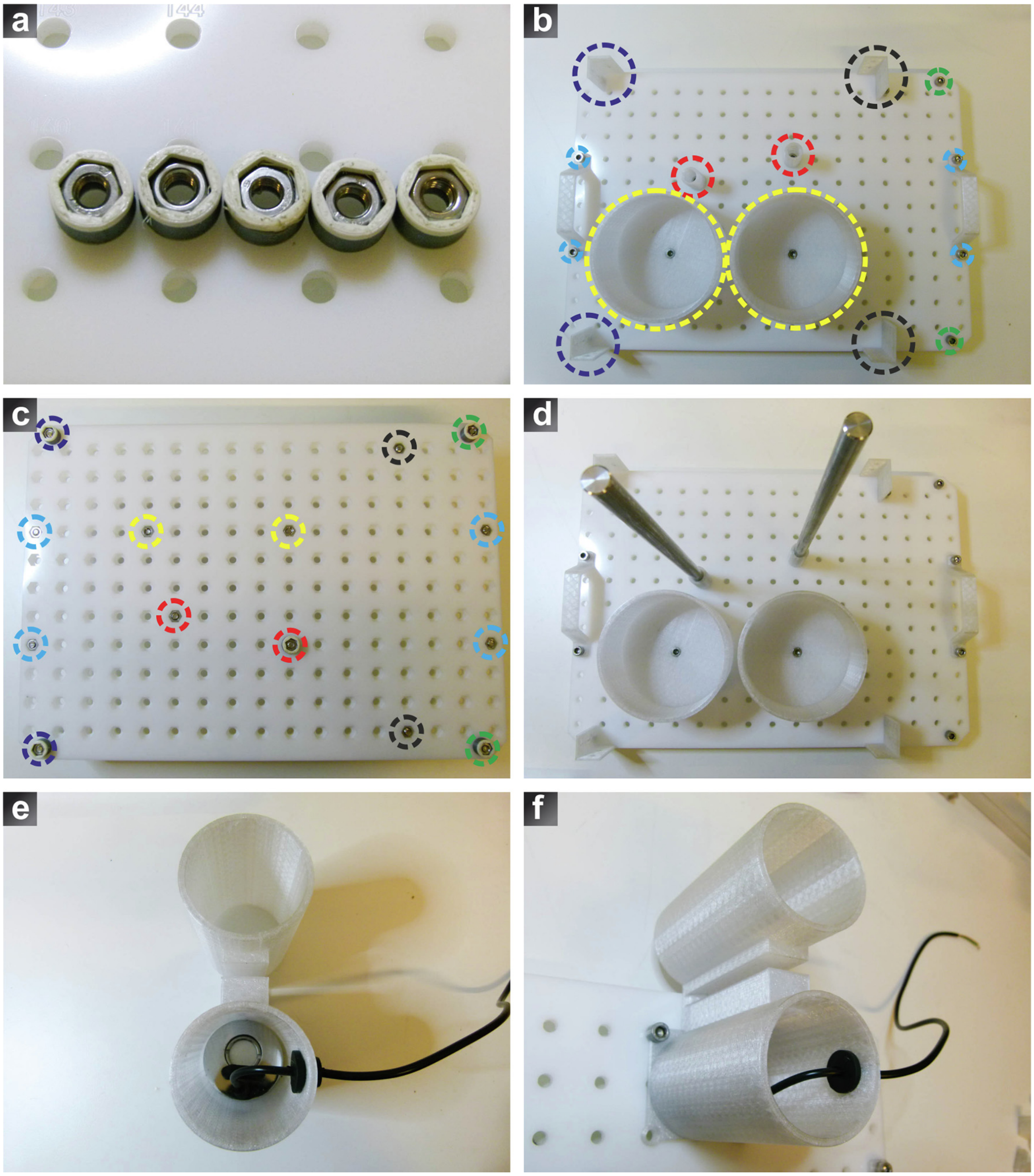


Fig. 2. Assembly of baseplate. (a) Insertion of M6 nuts into feet. (b) Arrangement of various holders on baseplate 1. (c) Arrangement of M6 nuts in baseplate 2. (d) Insertion of aluminum rods into rod holders. (e) Insertion of ultrasonic mist fogger in humidifier base. (f) Mounting of humidifier base on baseplate assembly.

5. Attach the two handles (*handle.stl*) to the baseplate by fastening them with 4 M6 $\times$ 14 hex head cap screws and 4 M6 nuts in slots 52 and 120 as well as 68 and 136 (Fig. 2b; blue highlights).
6. Next, attach the first aluminum rod holder (*rod_holder.stl*) to the baseplate by using an M6 $\times$ 25 hex head cap screw. Insert the screw into the holder and into slot 61 and fasten in from the bottom with the last foot in which an M6 nut was placed in step 1. Then attach the other rod holder to slot 74 using an M6 $\times$ 20 hex head cap screw and an M6 nut (Fig. 2b; red highlights).
7. Attach the two beaker holders (*beaker_fix.stl*) to the baseplate in slots 124 and 129 with the use of two M6 $\times$ 10 hex head cap screws and two M6 nuts (Fig. 2b; yellow highlights). Viewed from the top, the assembly should now look as in Fig. 2b and viewed from the bottom, it should look like in Fig. 2c.
8. Insert two 12 $\times$ 300 mm aluminum rods in the two rod holders (Fig. 2d).

9. Insert the ultrasonic mist fogger into the humidifier base (*humidifier_2.0.stl*) and lead the cable through the hole in the side as depicted (Fig. 2e).
10. Use two M6 × 15 hex head cap screws and two M6 nuts to attach the humidifier base to the baseplate in slots 11 and 47 (Fig. 2f).

5.2. Assembly of climate chamber

After cutting the box components (*box_back.dxf*, *box_doorside + electronics_cover.dxf*, *box_front.dxf*, *box_side.dxf*, *box_top + SSR_cover.dxf* and *door + frame.dxf*) for the climate chamber from sheets of polymer, prepare them for assembly by cutting the threads into the relevant holes as shown in Fig. 3.

The climate chamber box is designed to be assembled with press-fits by using the finger joints at the edge of the single components. Fig. 4 gives an overview about how the box has to be assembled. If the cutting parameters of the laser cutter are suitable for the tolerance specified in the designs, the assembled box should stably hold together only due to the press-fit.

To complete the assembly of the climate chamber box, follow the next steps:

1. Once the press-fit assembly of the box is completed (Fig. 5a), insert the box in between the box corners on top of the baseplate with the door cut-out of the box facing towards the direction of baseplate slots C and D. Then fasten the box to the box corners by using sixteen M3 × 10 hex head cap screws (Fig. 5b).
2. For increased rigidity, attach the four top corners (*corner_top.stl*) to the upper corners of the box with twelve M3 × 10 hex head cap screws (Fig. 5c).
3. Attach the laser-cut door frame to the door side of the box with sixteen M4 × 10 button head screws as shown in the picture (Fig. 5d).
4. Mount the female leaves of the two door hinges to the door frame with six M3 × 10 hex head cap screws (Fig. 5e). Then mount the male counterparts to the laser-cut door with six M3 × 6 hex head cap screws (Fig. 5f).
5. To attach the door lock to the door of the box, insert the component lock_hole-screw (*lock_hole-screw.stl*) into the remaining hole of the door from the side of the attached hinges and fasten it with the component lock_big-nut (*lock_big-nut.stl*) from the other side (Fig. 5g).
6. Assemble the lock with the missing components as shown in Fig. 5h.
7. Attach the door to the box by connecting the male and female leaves of the hinges with the use of two M3 × 25 hex head cap screws (Fig. 5i).

5.3. Assembly of peristaltic pump

To assemble the peristaltic pump, follow the next steps, which are illustrated in Fig. 6:

1. Insert an M3 nut into the nut trap of the bottom part of the roller pump head (*bearing_mount_bottom_01.stl*) (Fig. 6a). Then push three 4 × 14 mm straight pins into the cavities as shown (Fig. 6b).
2. Insert an M3 × 10 grub screw from the side and screw it into the M3 nut (Fig. 6c).
3. Mount three HK 0408 needle bearings on the straight pins (Fig. 6d).
4. Attach the top part of the roller pump head (*bearing_mount_top.stl*) to the bottom part using three M3 × 20 hex head cap screws (Fig. 6e).
5. Mount the Nema17 stepper motor to the housing (*pump_housing.stl*) by inserting four M3 × 10 hex head cap screws into the component pump_case_bottom (*pump_case_bottom.stl*) and fastening them through the holes in the housing into the threads of the stepper motor (Fig. 6f). The small hole in pump_case_bottom has to face upwards.
6. Mount the roller pump head onto the shaft of the stepper motor and fasten it by inserting the correct Allen key through the hole in the top of the component pump_case_bottom (Fig. 6g).
7. Insert two M3 × 25 hex head cap screws into the back of the component pump_case_top (*pump_case_top_120.stl*) and mount it onto pump_case_bottom. Then insert two M3 × 20 hex head cap screws into the remaining holes in the front (Fig. 6h).
8. After the assembly of the peristaltic pump has been finalized, attach the housing of the pump to the baseplate in the slots 16 and 50 with the use of two M6 × 15 hex head cap screws and two M6 nuts. The roller pump head faces outward from the baseplate assembly (Fig. 6i).

5.4. Assembly of heat lamp housing

To assemble the housing for the ceramic infrared heat lamp, refer to Fig. 7 and follow these steps:

1. Cover the entire inner surface of the lamp housing (*lamp_case.stl*) with aluminum tape (Fig. 7a).
2. Install the ceramic power socket into the lamp housing (Fig. 7b).
3. Mount the lamp housing on top of the climate chamber with the use of six M6 × 12 hex head cap screws (Fig. 7c).

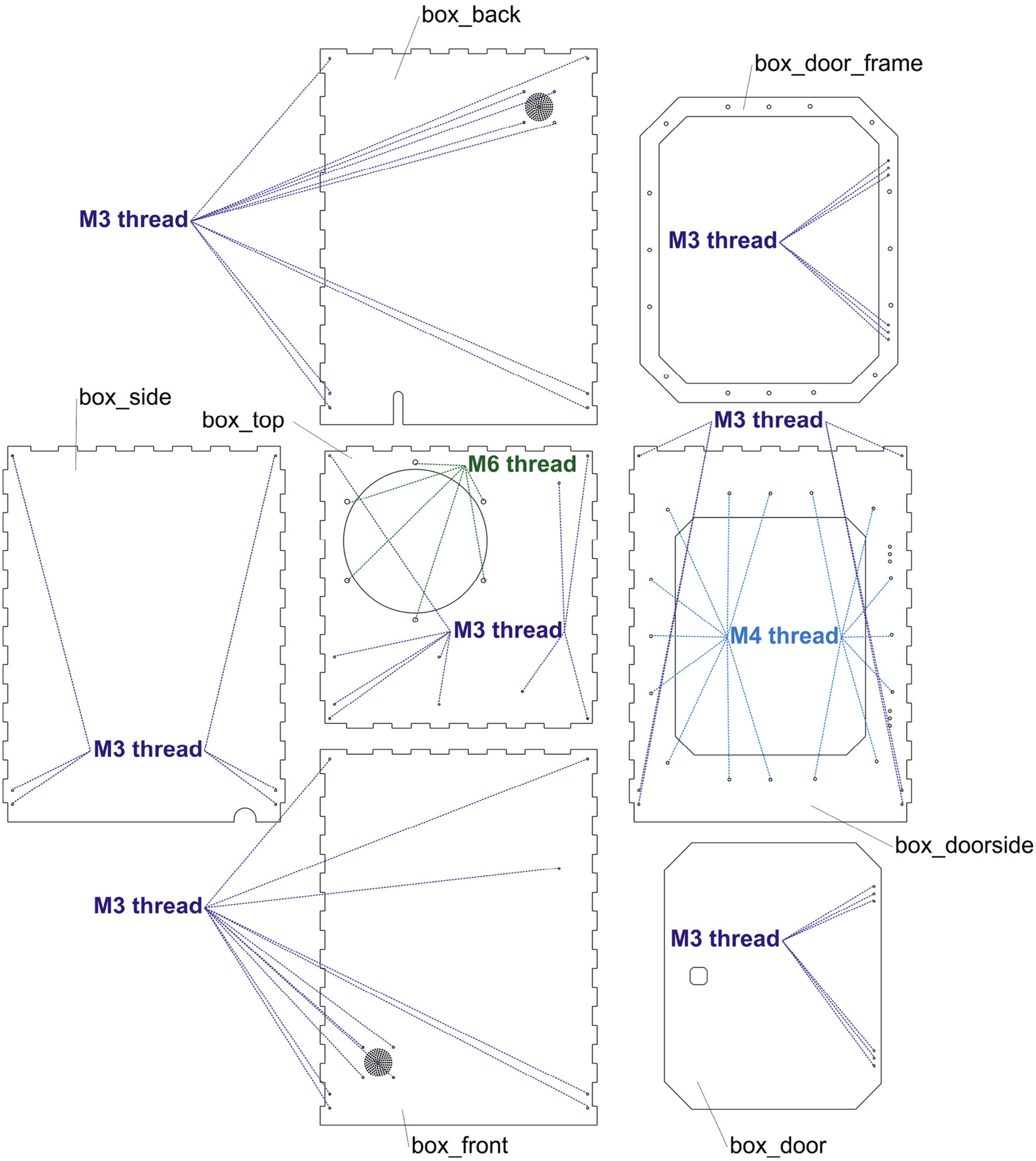


Fig. 3. Threading instructions for preparation of box elements for climate chamber.

5.5. Assembly of display controller

The assembly of the display controller is illustrated in Fig. 8.

1. Push the shaft of the rotary encoder from the inside of the controller housing (*controller_housing.stl*) through the hole in the side and fasten it with the supplied nut. Then press-fit the knob for the shaft (*controller_knob.stl*) onto the shaft (Fig. 8a). Note that it is required that the rotary encoder possesses a 10kΩ pull-up resistor in the highlighted location.

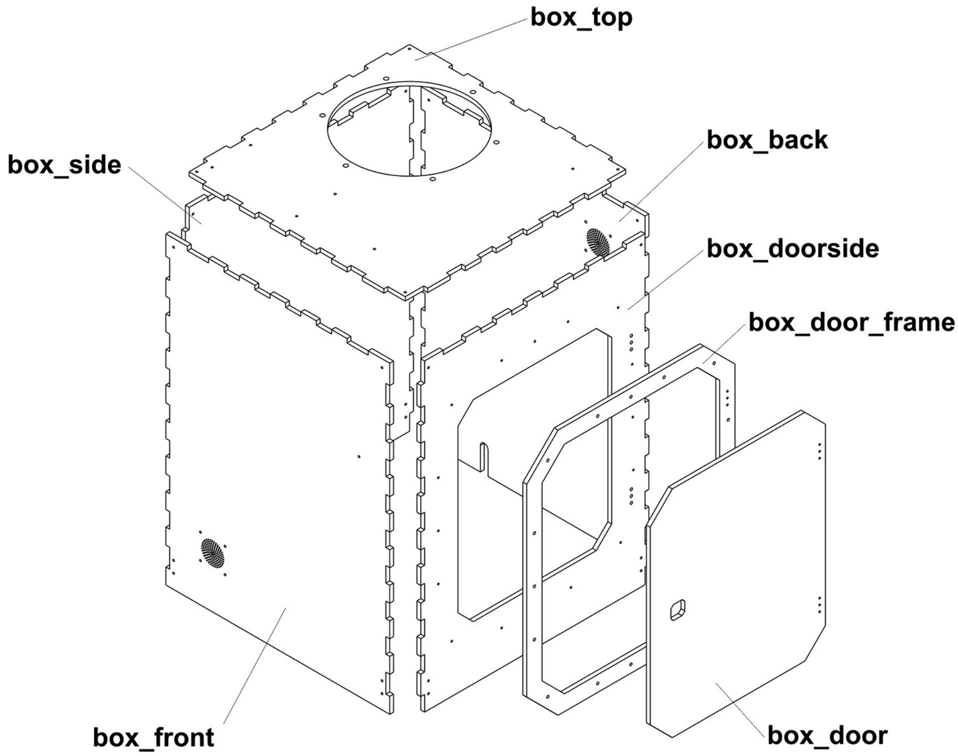


Fig. 4. Exploded view of climate chamber assembly.

2. Insert the I2C LCD into the cavity of the controller housing and fasten it with four M3 × 16 hex head cap screws and four M3 nuts. To not damage the printed circuit board (PCB), insert some plastic spacers. You can 3D print the plastic washers from the supplied design (*plastic_washers.stl*). Connect the cables to the rotary encoder and the LCD and feed them through the hole in the back. You can tie the cables together with a zip tie for strain relief (Fig. 8b). The completed housing should look like depicted in Fig. 8c.
3. Finally, attach the display controller to the climate chamber with the use of four M3 × 8 hex head cap screws (Fig. 8d).

5.6. Lamp assembly

Three IKEA ledberg LED spots serve as a light source for the retention model setup. The assembly of the lamp is depicted in Fig. 9. Follow these steps to complete the assembly:

1. Insert the three LED spots in the lamp housing (*ikea_led_housing.stl*) and feed the cables through the channels at the sides of the cavities (Fig. 9a). You can use the supplied adhesive to hold the spots in the housing.
2. The LED spots are usually distributed in a pack of four, however for this purpose only three are required. The four LED spots are connected to power with a small PCB hub (Fig. 9b). You can shorten the cables, de-solder the fourth LED spot and re-solder the rest of them. Finally, you can use one of the supplied adhesives to glue the hub on top of the climate chamber (Fig. 9c).

5.7. Mounting sensors

The climate control (temperature and humidity) in the climate chamber uses input signals of two DHT22 temperature and humidity sensors. Each of them is placed differently in the chamber. While one of them measures the ambient temperature and humidity in the chamber, the other one measures local temperature and humidity at the slide holder, next to the slide where the tissue is placed. The instructions to mount the sensors are illustrated in Fig. 10 and are summarized in the next steps:

1. Insert an M6 nut and an M6 × 12 hex head cap screw into the slide holder (Fig. 10a).
2. Mount the local sensor (T/H sensor 1) to the sensor holder on the slide holder with an M3 × 8 hex head cap screw (Fig. 10b).

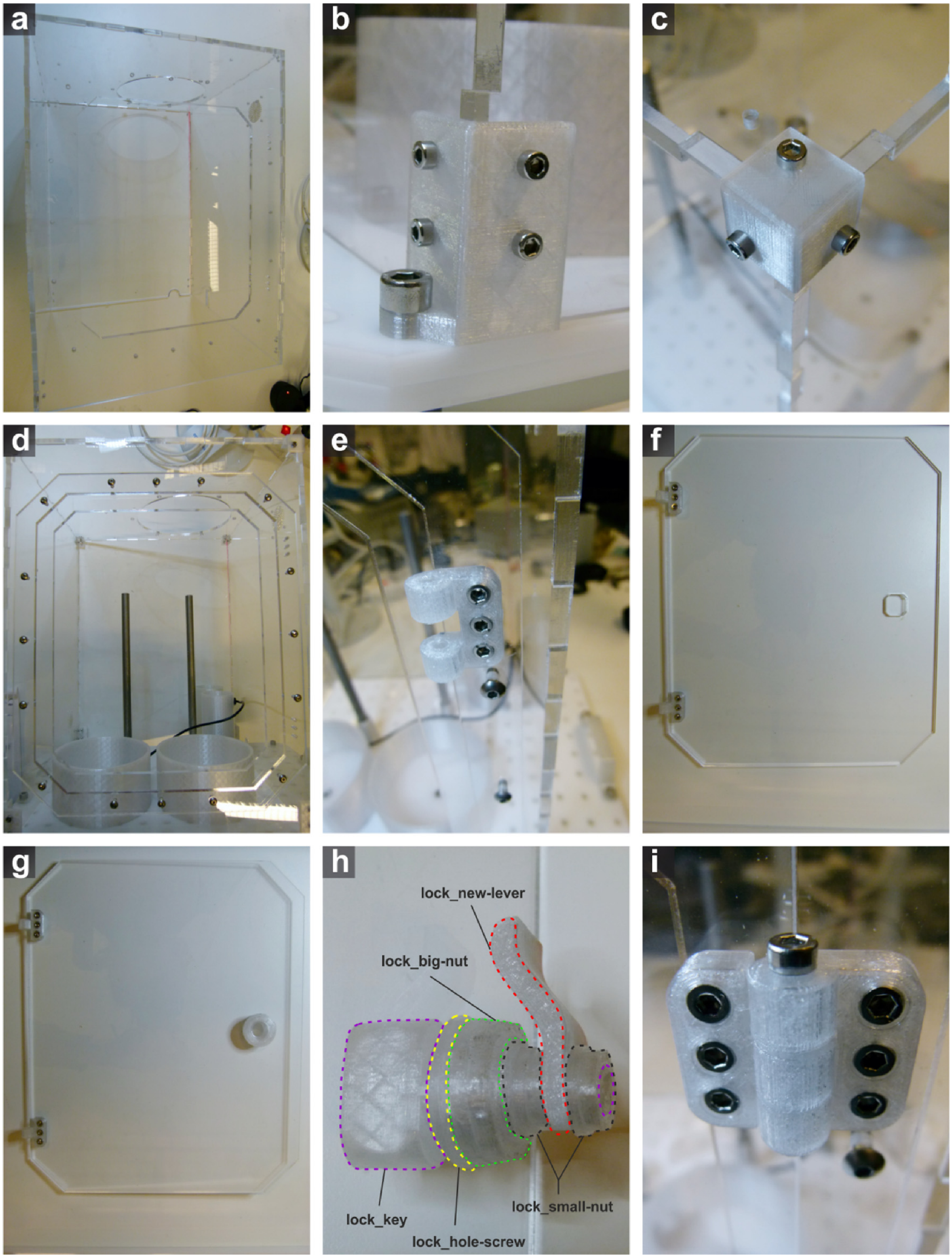


Fig. 5. Climate chamber assembly. (a) Finished press-fit assembly of acrylic box. (b) Fastening of acrylic box to 3D printed corners. (c) Mounting of box top corners. (d) Attachment of door frame to acrylic box. (e) Mounting of female hinge leaves on door frame. (f) Mounting of male hinge leaves on acrylic door. (g) Insertion of hole screw for door lock. (h) Assembly of door lock. (i) Assembly of door hinges with M3 × 25 screws.

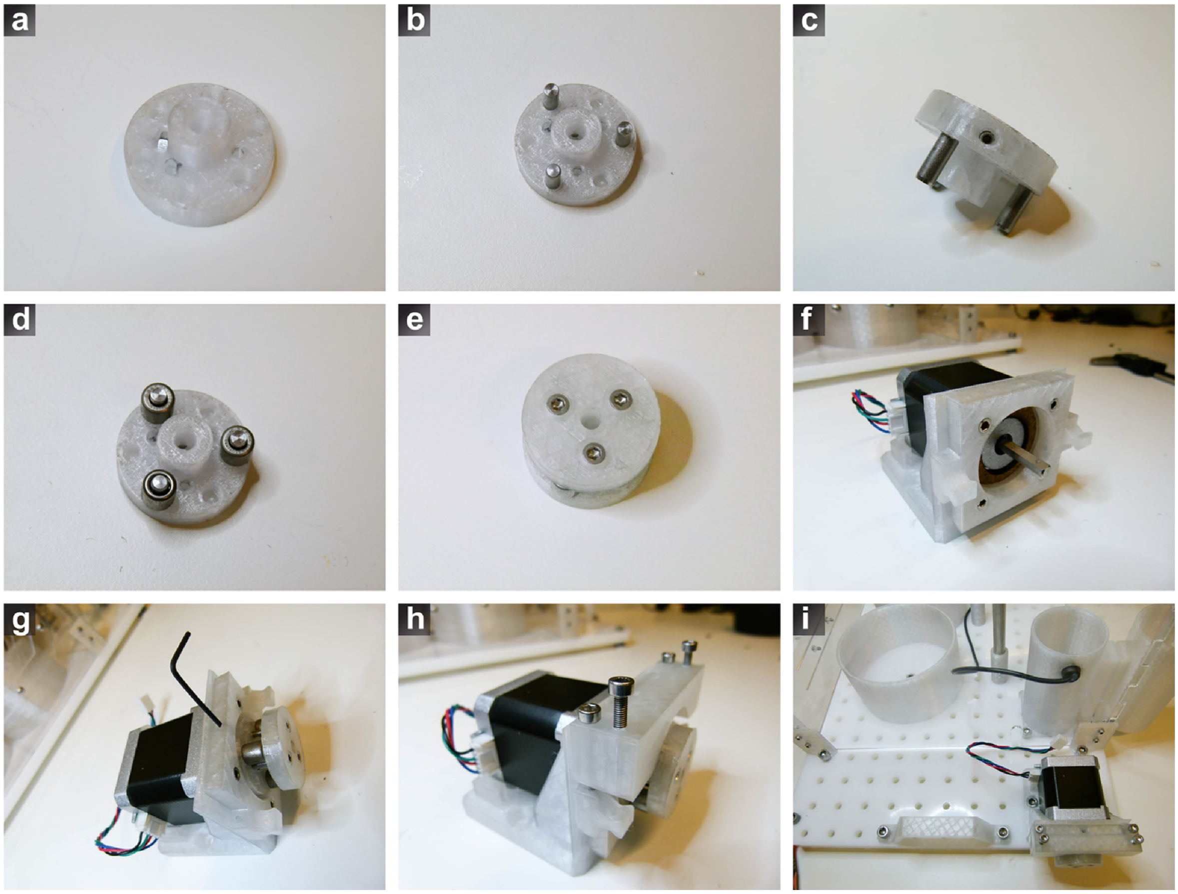


Fig. 6. Assembly of peristaltic pump. (a) Insertion of M3 nut into nut trap. (b) Insertion of 4 × 14 mm straight pins. (c) Installation of M3 × 10 grub screw into M3 nut. (d) Mounting of needle bearings. (e) Assembly of roller pump head. (f) Mounting of Nema17 stepper motor. (g) Installation of roller pump head. (h) Final assembly of pump. (i) Mounting peristaltic pump to baseplate.

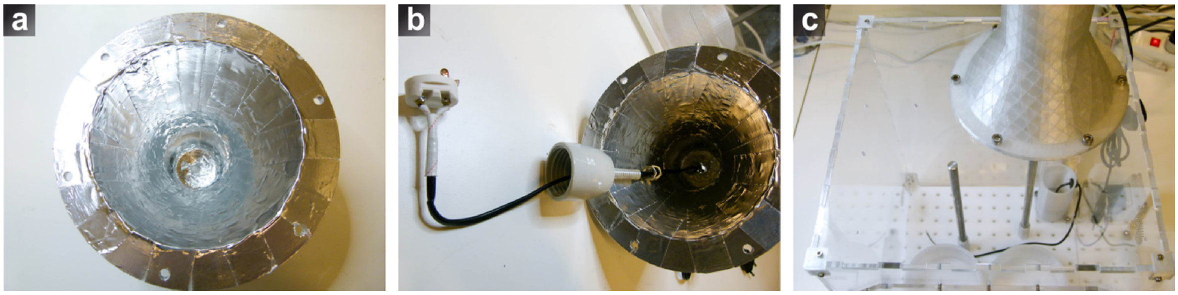


Fig. 7. Assembly of case and power socket for infrared heat lamp. (a) Covering of lamp housing with aluminum tape. (b) Installation of ceramic power socket. (c) Mounting of lamp housing on climate chamber with six M6 × 12 hex head cap screws.

3. Mount the ambient sensor (T/H sensor 2) from the inside to the front of the climate chamber with an M3 × 8 hex head cap screw and mount the slide holder onto the aluminum rod, which is closer to the back of the climate chamber (Fig. 10c).

5.8. Installation of fans and cable routing

Temperature and humidity in the climate chamber are regulated, among others, with the use of three 12 V DC fans. One fan acts as a venting fan that blows air from the outside into the chamber. A second fan acts as an exhaust fan, dragging air out of the chamber. The last fan is placed on the humidifier to distribute the steam, which is generated by the ultrasonic mist fogger. Refer to Fig. 11 for the installation of these fans.

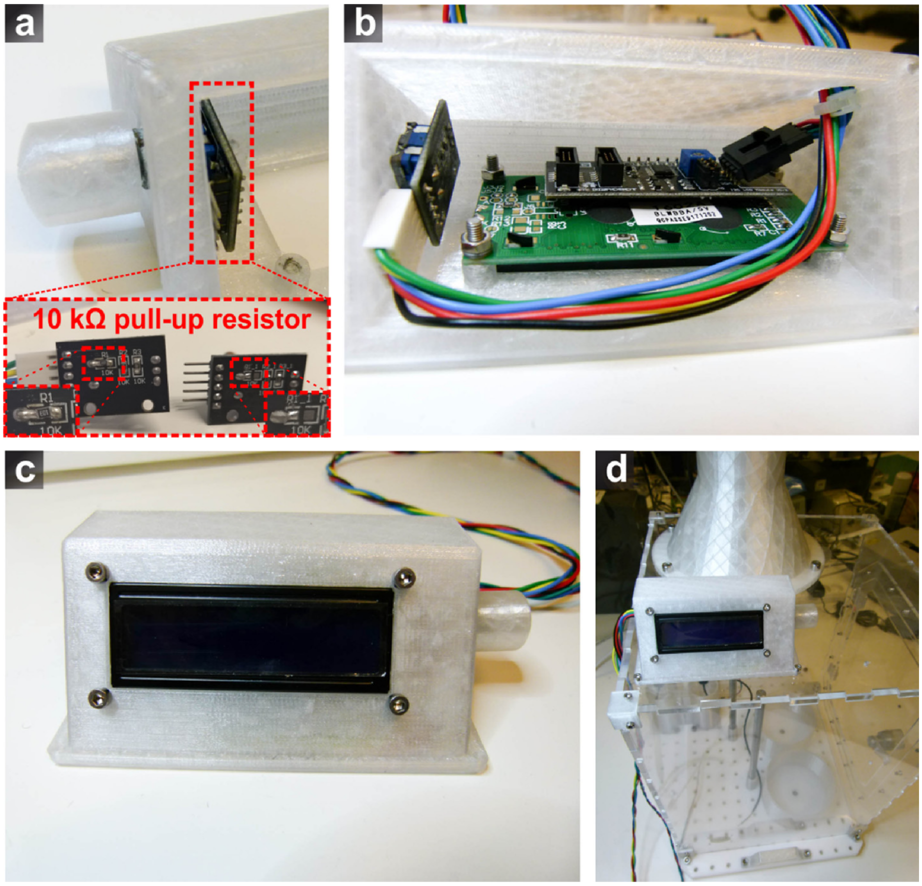


Fig. 8. Assembly of controller housing with rotary encoder and I2C LCD display. (a) Installation of rotary encoder and controller knob. Note that the rotary encoder needs to exhibit a 10 kΩ pull-up resistor in the labeled position. (b) Insertion and fastening of I2C LCD with four M3 × 16 hex head cap screws, four plastic washers and four M3 nuts to controller housing. (c) Front view of assembled controller. (d) Mounting of controller on top of climate chamber with four M3 × 8 hex head cap screws.

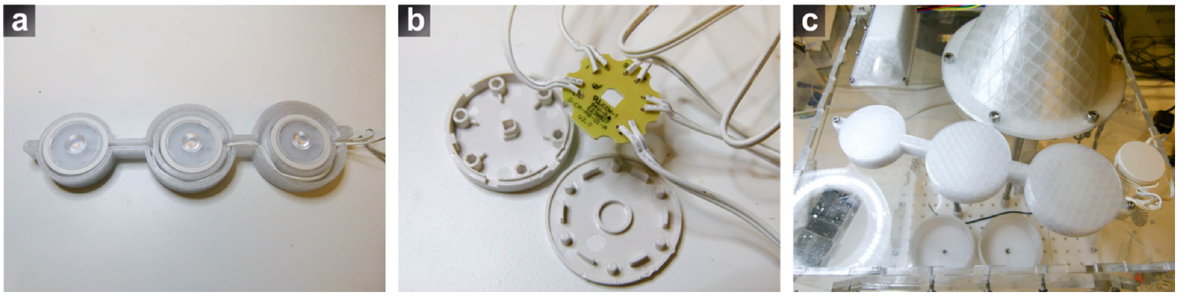


Fig. 9. Assembly of IKEA Ledberg LED lighting. (a) Insertion of LED spots into 3D printed housing. (b) PCB connector hub. (c) Mounting of lamp housing on top of climate chamber.

1. The venting fan is mounted along with a fan grill (*40mm_Fan_grill_final.stl*) to the front of the climate chamber by using 4 M3 × 30 hex head cap screws (Fig. 11a). Note that the direction of air flow should be towards the climate chamber. If the fan blades are too close to the fan grill, you can use the 3D printed plastic washers (*plastic_washers.stl*) to increase the spacing.
2. The exhaust fan is as well mounted together with a fan grill but attached to the back of the climate chamber (Fig. 11b). Here, the direction of airflow should be away from the chamber.

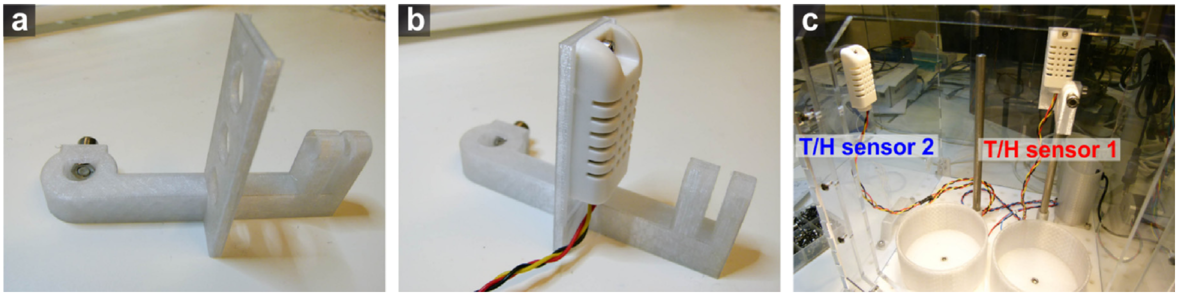


Fig. 10. Installation of DHT 22 temperature and humidity sensors. (a) Insertion of M6 nut and M6 × 12 hex head cap screw into slide holder. (b) Mounting of first DHT22 temperature and humidity sensor to slide holder with an M3 × 8 hex head cap screw. (c) Attachment of second DHT22 sensor to front of climate chamber.

3. You can route the cables of the venting fan and the two humidity sensors through the baseplate to the back of the climate chamber as it is depicted (Fig. 11c). At this point, you can also already pre-install the cable for the motor of the autosampler.
4. Place a mesh (*humidifier_mesh.stl*) that hinders water splashes to hit the fan of the humidifier (*humidifier_2.0.stl*) into the humidifier cavity that holds the ultrasonic mist fogger (Fig. 11d).
5. Install the humidifier fan (40 × 10 mm) in a "sandwich" with two fan grills to the cap of the humidifier (*humidifier_fan.stl*) by using four M3 × 30 hex head cap screws (Fig. 11e) and then stack the cap onto the humidifier base (Fig. 11f).

5.9. Autosampler assembly

For the facilitation of automated experiments and thereby increased reproducibility, the presented retention model setup features a fully 3D printed rotational autosampler. Execute the assembly of the autosampler as described (Fig. 12).

1. Start the assembly by attaching the motor mount (*geardrive_motormount.stl*) to the planetary gear drive (*planetary_gear_-drive.stl*) with one screw connection by using one M3 × 6 countersunk screw as highlighted (Fig. 12a).
2. Insert the Nema17 stepper motor into the cavity of the motor mount and fasten it with four M3 × 6 countersunk screws (Fig. 12b). As the motor mount is attached to the planetary gear drive with only one screw, you can rotate the planetary gear drive to obtain access to all screws.
3. Insert an M3 nut into the nut trap of the small herringbone gear (*herringbone_gear.stl*) (Fig. 12c).

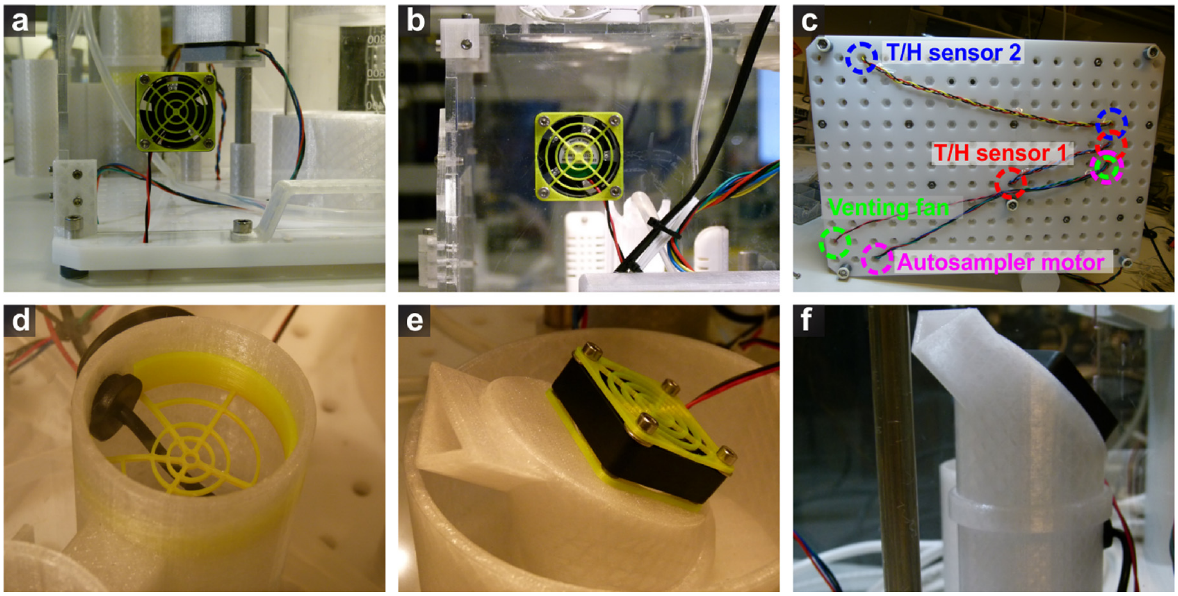


Fig. 11. Installation of fans with four M3 × 30 hex head cap screws. (a) Installation of venting fan and fan grill to the front of the climate chamber. (b) Mounting of exhaust fan in the same way to the back of the climate chamber. (c) Cable routing at the bottom of the baseplate. (d) Insertion of protection mesh into humidifier base. (e) Mounting of fan and two fan grills to the humidifier cap. (f) Installation of humidifier cap on humidifier.

4. Install the small herringbone gear onto the shaft of the stepper motor with one M3 × 10 hex head cap screw as highlighted (Fig. 12d).
5. Fix the motor mount to the planetary gear drive with three more M3 × 10 hex head cap screws (Fig. 12e).
6. Insert an M6 nut and M6 × 10 hex head cap screw into the motor mount as highlighted (Fig. 12f).
7. Finalize the assembly by attaching the cover (*gear_drive_cover.stl*), the shim (*planetary_gear_drive_shim.stl*) and the ring gear (*ring_gear.stl*) to the planetary gear drive and each other with four M3 × 25 countersunk screws as shown in the picture (Fig. 12g). Also, insert two M3 × 8 grub screws into the cover of the geardrive.
8. Mount the assembled autosampler on the aluminum rod in the front of the climate chamber (Fig. 12h).
9. Mount a suitable sample holder onto the autosampler (in this case *mesh_holder.stl* and *small_mesh.stl*; several holder designs can be found in the Mendeley data repository) and secure it with 3D printed thumb nuts (*thumbnut.stl*) (Fig. 12i). When 3D printing the thumbnuts, stop the print at the height of the end of the nut trap and insert an M3 nut, then continue the 3D print.

5.10. Assembly and wiring of solid-state relay

As the ceramic heat lamp for temperature-control needs to be connected to the *power line with high voltage* and to the Arduino through a solid-state relay, the solid-state relay is placed separated from all other components for safety reasons. Always refer to a professional to make sure that the connections are correct and safe. Additionally, a fuse is installed to prevent electrical accidents. To install and wire the solid state relay, refer to Figs. 13 and 14, respectively.

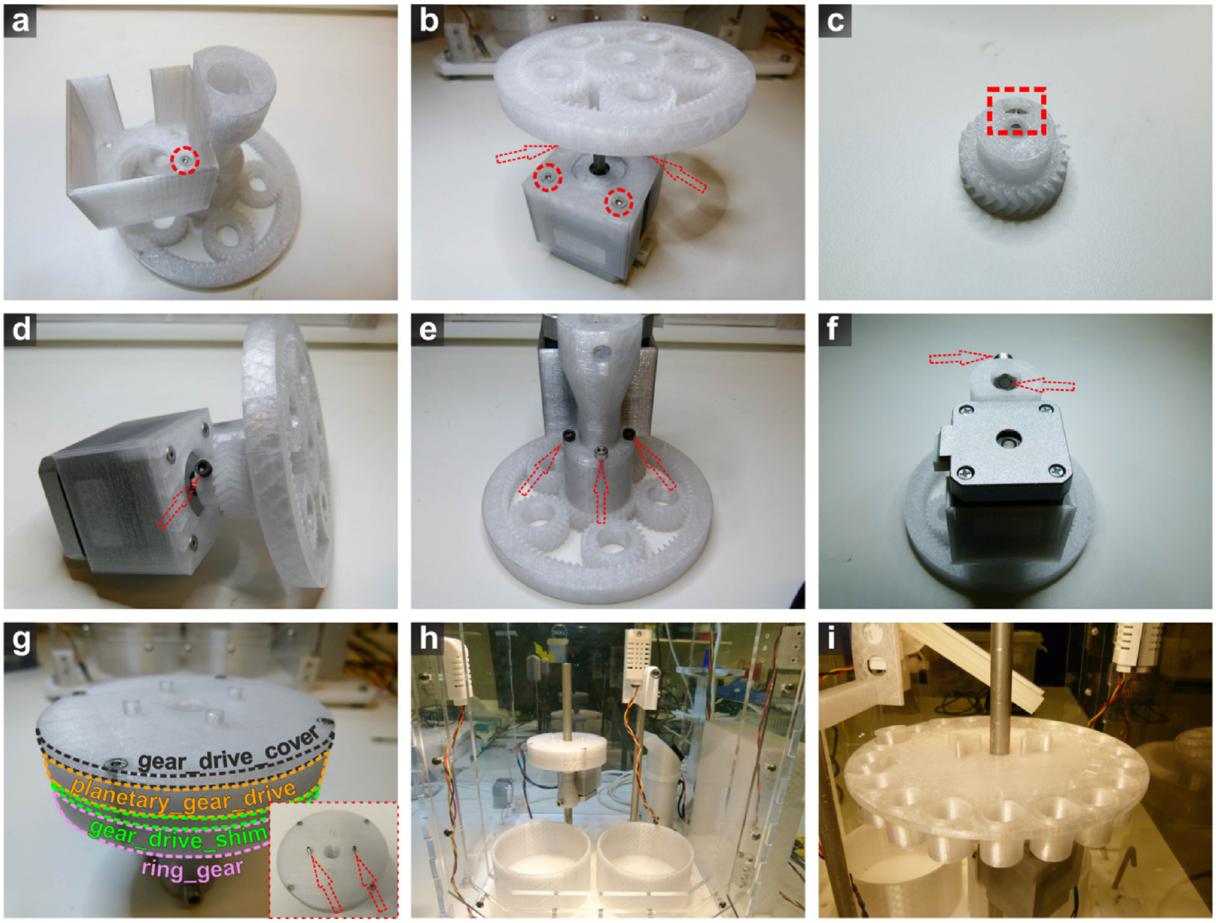


Fig. 12. Assembly of motorized rotary autosampler. (a) Connection of motor mount and planetary gear drive with one M3 × 6 countersunk screw. (b) Mounting of Nema17 stepper motor with four M3 × 6 countersunk screws. (c) Insertion of M3 nut into herringbone gear. (d) Mounting of herringbone gear on motor shaft with one M3 × 10 hex head cap screw. (e) Final fastening of motor mount to planetary gear drive with three M3 × 10 hex head cap screws. (f) Insertion of M6 nut and M6 × 10 hex head cap screw into motor mount. (g) Sandwich-style assembly of autosampler elements with four M3 × 25 countersunk screws. Inlet shows that two M3 × 8 grub screws are inserted into the top of the gear drive cover. (h) Installation of completed autosampler on aluminum rod in retention model setup. (i) Setup with sample holder and fastening with two thumb nuts.

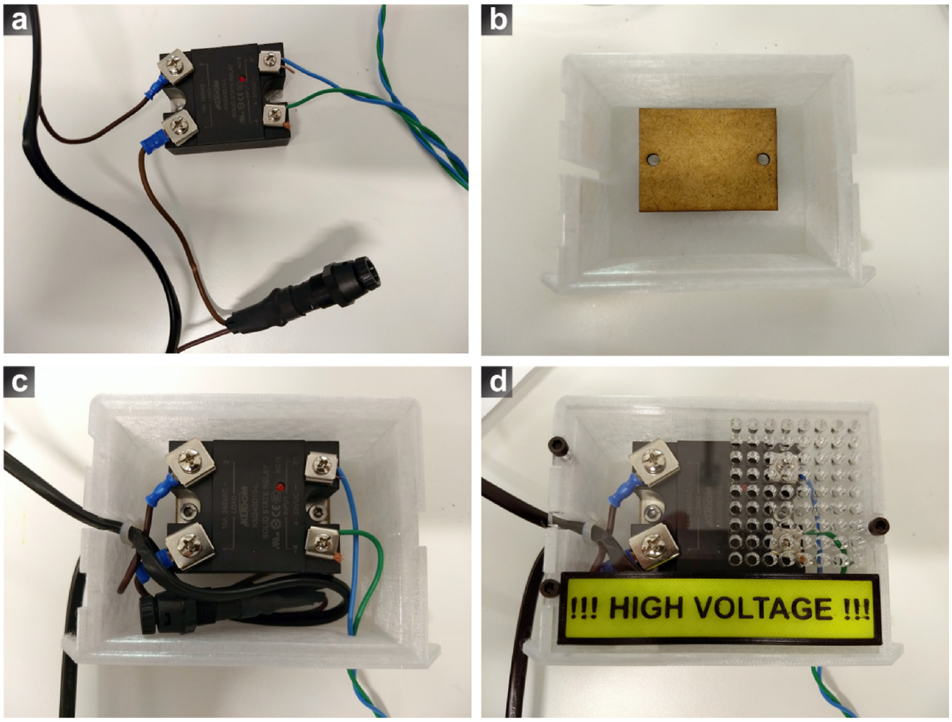


Fig. 13. Assembly of solid-state relay in solid-state relay housing. (a) Connection of solid-state relay with fuse holder and power socket of the heat lamp. (b) Insertion of wooden protection plate. (c) Placing of solid-state relay and fuse holder in housing. (d) Installation of acrylic cover and warning sign.

Fig. 14. Wiring of solid-state relay to fuse, heat lamp and RAMPS 1.4 Arduino shield.

1. Splice out one wire of the cable from the ceramic heat lamp power socket and connect it to the inline fuse holder and the load connectors of the solid-state relay (Fig. 13a). Also, prepare two wires for the input signal of the relay. Finally, insert a 3A cartridge fuse into the fuse holder.
2. Insert the laser-cut wooden plate (*woodplate_ssr.dxf*) into the housing (*ssr_housing.stl*), so that the holes in the plate are aligned with the holes in the housing (Fig. 13b).
3. Attach the solid-state relay to the housing by fastening it with two M4 × 8 hex head cap screws trough the wooden plate to the housing (Fig. 13c). Feed the cable of the heat lamp power socket through the slot at the side and the wires for the input signal through the hole in the bottom. Place the fuse holder next to the relay and use zip ties for strain relief.
4. Finally, close the housing with an acrylic cover (from *box_top + SSR_cover.dxf*) by fastening it with three M3 × 10 hex head cap screws and apply the warning sign (*warning_sign.stl*) with adhesive tape (Fig. 13d). You can print the warning sign with different colors to increase visibility.

5.11. Assembly of electronics and wiring

The installation and wiring of all electronic components inside the electronics housing (*electronics_housing.stl*) is illustrated in Figs. 15–17.

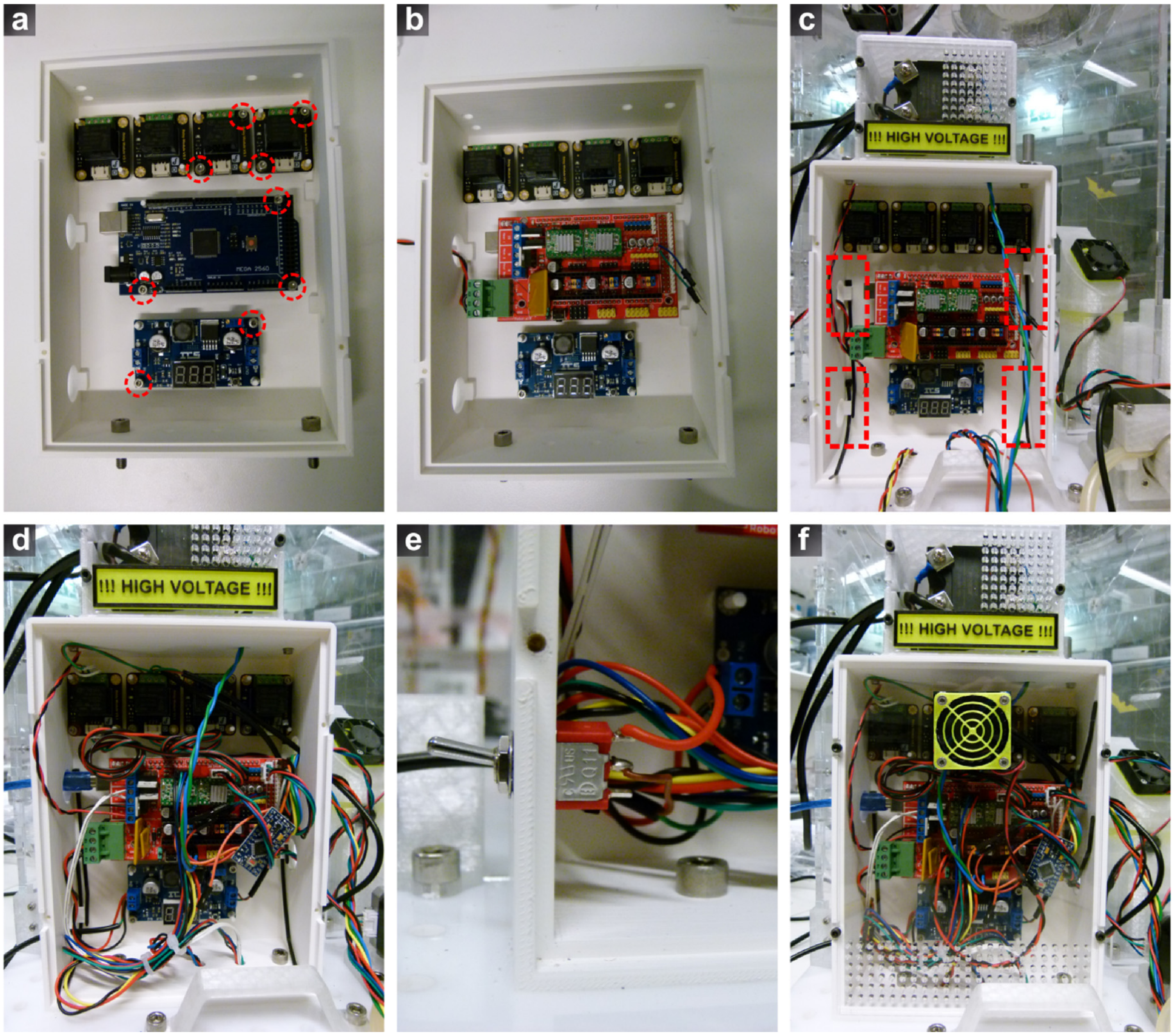


Fig. 15. Assembly of electronic components in electronics housing. (a) Placement of relays, Arduino and DC/DC boost converter in electronics housing and fastening with M3 × 8 hex head cap screws. (b) RAMPS 1.4 Arduino shield stacked on top of Arduino. (c) Mounting of electronics housing on baseplate with two M6 nuts and two M6 × 20 hex head cap screws and of solid-state relay housing on electronics housing with two M6 × 10 hex head cap screws. The picture also shows how the cables are routed into the electronics housing. (d) Connection of cables to RAMPS 1.4 Arduino shield. (e) Installation and connection of power switch. (f) Attachment of acrylic cover with four M3 × 10 hex head cap screws and mounting of fan with four M3 × 10 hex head cap screws.

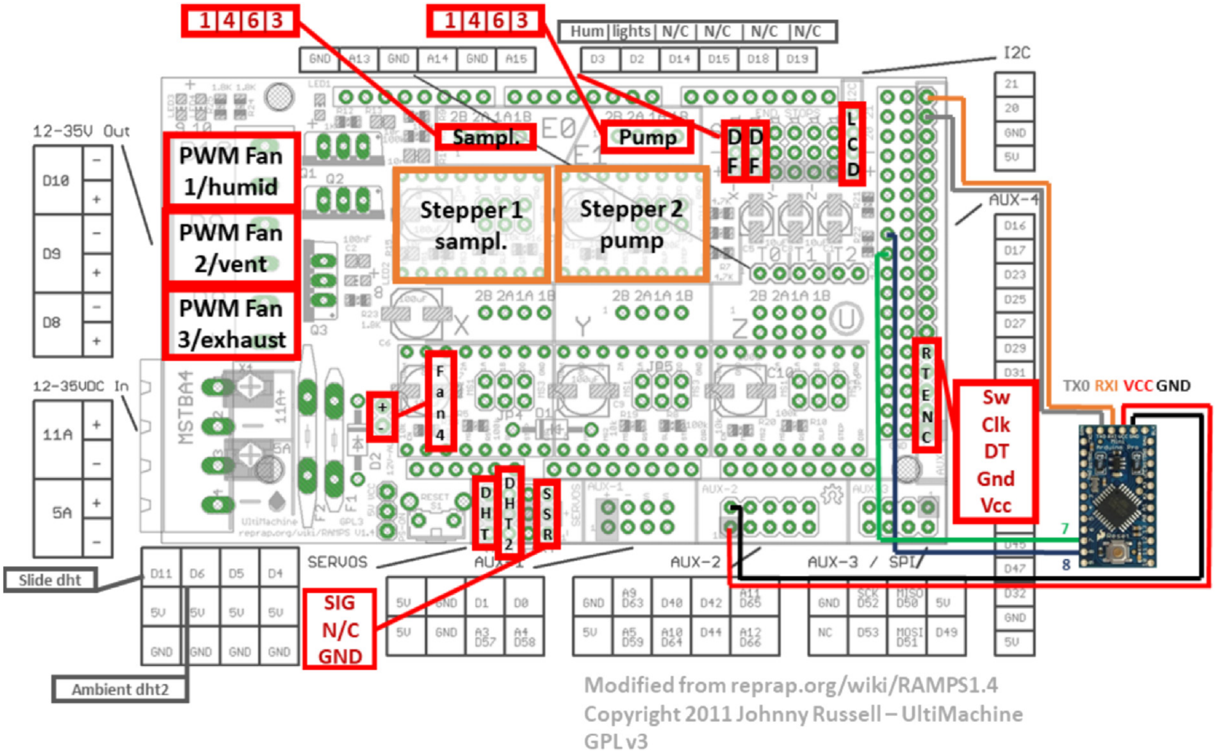


Fig. 16. Wiring of electronic components to RAMPS 1.4 Arduino shield.

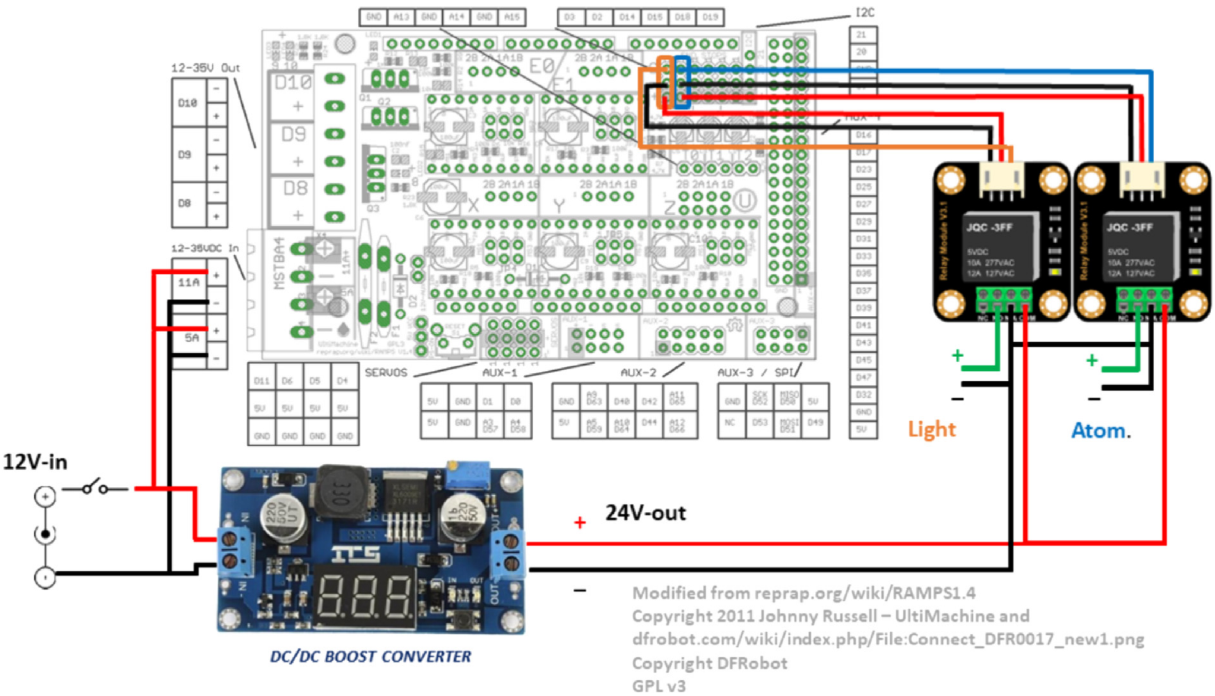


Fig. 17. Wiring of power supply, DC/DC boost converter and relays.

1. Place the two required relay boards on the pins in the electronics housing (there are two expansion slots). Also, place the Arduino Mega 2560 and the DC/DC boost converter and fasten all components down with nine M3 × 8 hex head cap screws in the highlighted positions (Fig. 15a). Furthermore, insert two M6 × 20 hex head cap screws in the bottom of the housing.
2. Solder two short wires for the Arduino Pro Mini to the RAMPS1.4 shield and stack it onto the Arduino afterwards (Figs. 15b, 16).
3. Fasten the electronics housing to the baseplate of the retention model setup with two M6 nuts in slots 83 and 151 and route the wires into the housing as depicted (Fig. 15c). You can insert zip ties in special slots in the housing (highlighted) to tie the wires together. Feed the input signal wires from the solid-state relay through the hole in the top of the electronics housing and attach the with two M6 × 10 hex head cap screws.
4. Connect all wires and an Arduino Pro Mini (upload sketch *mini_pump_control.ino* beforehand) to the RAMPS1.4 shield according to the wiring schemes in Figs. 16 and 17 (Fig. 15d). Connect and mount a power toggle switch as well (Fig. 15e).
5. Finally, attach the last fan with fan grill to the acrylic cover (from *box_doorside + electronics_cover.dxf*) with four M3 × 30 hex head cap screws and flow direction towards the acrylic. Feed the fan wires through the hole in the acrylic and connect the fan (Fig. 16, Fan4). Then screw the cover to the housing with use of four M3 × 10 hex head cap screws.

5.12. Insertion of tubing and final setup

To finalize the construction of the retention model setup, insert a peristaltic pump/lab silicone tubing with an inner diameter of 4 mm and a wall thickness of 1.6 mm into the peristaltic pump and attach the ends to the slide (tissue holder) and a beaker (Fig. 18). The 3D printable mesh (*mesh.stl*) possesses a hole with which the tube can be held in place.

A fully constructed retention model setup is depicted in Fig. 19.

6. Operation instructions

6.1. Structure of Arduino sketch for control with Mega 2560

In this section, the structure of the code for the Arduino Mega 2560 microcontroller (*main.ino*) is explained. At each iteration of the "main loop" the microcontroller handles 3 tasks:

- Menu navigation:

If an input from the user is detected (encoder rotated or clicked) the values of the "menu state variables" are modified and menu items are displayed accordingly. By navigating the menu as described in section 6.2, the user can manually enable/disable each of the functionalities of the system and configure all the parameters of the experiment.

- Climate-control:

The control of temperature and humidity is disabled by default and the display shows "Temp/Hum control off". If enabled (manually through menu navigation or within the automated program), the value of the temperature from the slide sensor (T) and the value of the humidity from the ambient sensor (H) are read and compared to the *setpoints* giving $\Delta T = T - target\_T$ and $\Delta H = H - target\_H$, respectively. Then the ambient control devices are actuated depending on the values ΔT and ΔH:

- if $\Delta H < 0$, the humidifier is turned ON by setting the digital signal driving the relay to high
- if $\Delta H < 0$, the humidifier fan is turned ON and its speed is controlled by a PWM (pulse width modulation) signal with a duty cycle proportional to ΔH

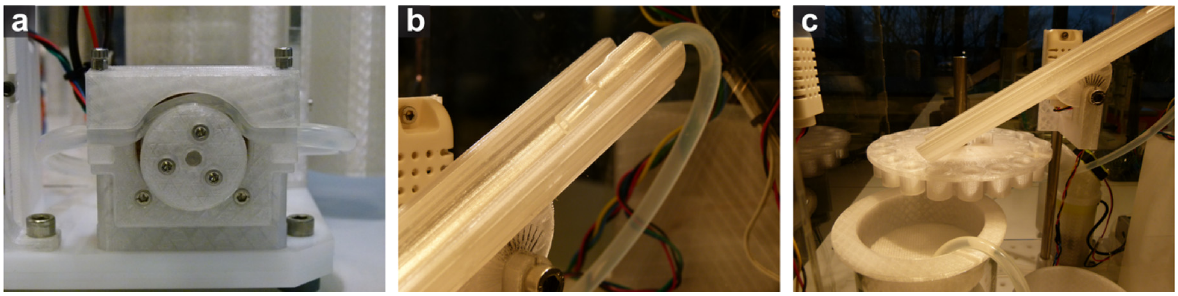


Fig. 18. Installation of peristaltic pump tubing. (a) Tube inserted into peristaltic pump. (b) Tube connection to slide. (c) Tube entering a beaker through a 3D printed mesh.

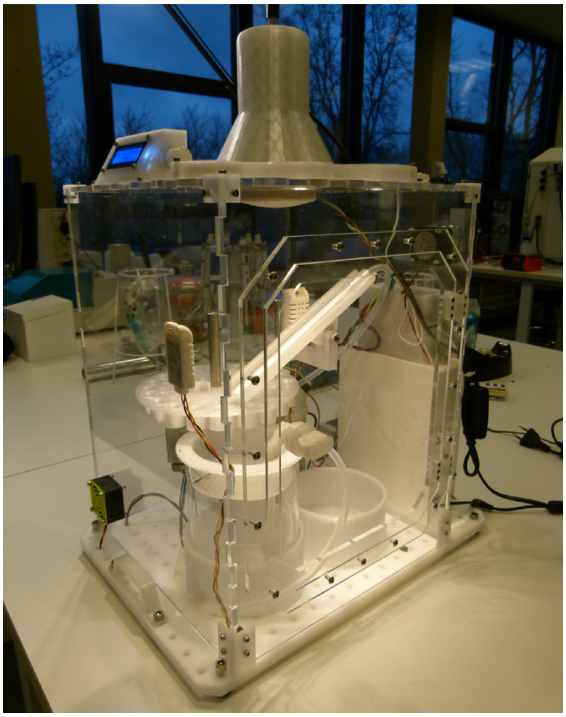


Fig. 19. Final setup of retention measurement system.

o if $\Delta T < 0$, the infrared heat lamp is turned ON by setting the digital signal controlling the solid-state relay to high
o if $\Delta H > 0$ or $\Delta T > 0$, the ventilation and exhaustion fans are turned ON and their speeds are controlled by PWM signals with duty cycles which are dependent on both ΔH and ΔT.
o Finally, the temperature of the ambient sensor (*amb_T*) is measured: if *amb_T* > 45 °C or the temperatures measured by the two sensors differ >15 °C (meaning that at least one of the sensors is not working properly) all the devices are turned OFF and the system stays in an ERROR state until it is restarted.

- Automated program execution:

If the automated program function is enabled, a single instruction of the automated program is executed (a more detailed description of the automated program is proposed in Section 6.3).

6.2. Structure of LCD/rotary encoder controller menu

The menu is structured in four layers, identified by different colors in Fig. 20: *Home* → *layer1* → *layer2* → *parameters*. By default, the LCD displays one of two possible home pages, depending on whether the ambient control is disabled ("*temp hum control OFF*") or enabled (displaying temperature and humidity values).

By clicking the push-button of the rotary encoder, the user enters the menu navigation mode: the first row of the LCD displays the current section of the menu while the second row displays the subsections/items of that section. By rotating the button, the users can scroll through the items and by clicking the push-button the user selects the displayed item and enters the corresponding subsection.

The current state of the menu navigation is uniquely identified by a pair of variables: *P* and *C* (Fig. 20). In the last layer of subsections (green blocks) the first row of the LCD displays the name of the parameter and the second row displays the current value of the selected parameter: the user can scroll through the possible values of the parameter by rotating the button and as the push-button is clicked the selected value of the parameter is stored and the menu moves back to the previous subsection.

Special cases:

- *Sel func* → *temp/hum control* → *enable*: by clicking the push-button while the "*enable*" item in the "*temp/hum control*" section is displayed, the ambient control will be enabled if it was previously disabled and disabled if it was previously enabled.

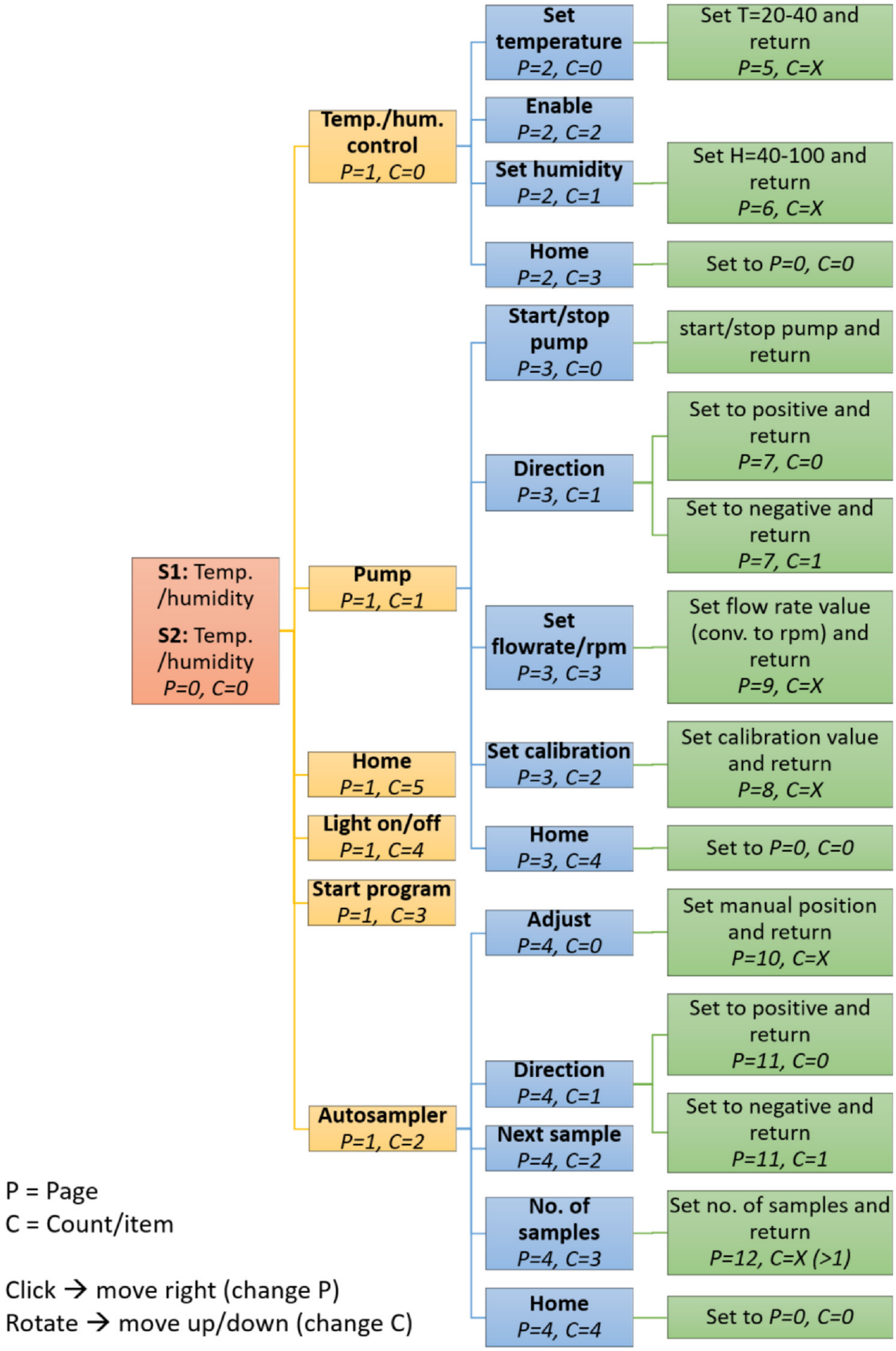


Fig. 20. Organigram of all menu points in the controller menu. Different colors correspond to different layers of the menu structure.

- *Sel func* → *pump* → *start/stop*: by clicking the push-button while the "*start/stop*" item in the "*pump*" section is displayed, the pump will be turned ON if it was previously OFF and turned OFF if it was previously ON.
- *Sel func* → *autosampler* → *next sample*: by clicking the push-button while the "*next sample*" item in the "*autosampler*" section is displayed, the autosampler will move to the next sample right away.

- *Sel func* → *autosampler* → *manual pos* → *X*: by rotating the knob while in the position parameter of the "*manual pos*" item of the "*autosampler*" section, the autosampler will move according to the rotation of the button to fine-tune the position of the autosampler. The user can exit the manual positioning mode by clicking the push-button at any point and the menu will return to the autosampler section.
- *Sel func* → *prog start/stop*: by clicking the push-button while the "*prog start/stop*" item of the "*sel func*" section is displayed, the automated program algorithm will start if it was previously disabled or it will stop if it was running.

By clicking on the "*Home*" item, present in any section and subsection, the user will exit the menu navigation and the LCD will display the home page.

6.3. Instructions for generation of customized automated program sequences

In order to build a custom designed automated program, the user must modify the "*my_program ()*" function that can be found at the very end of the Arduino code (*main.ino*). The "*my_program*" function is a case structure in which each case corresponds to one step of the desired sequence of operations. Each step (case) must contain only one instruction from the provided instruction set (Fig. 21). The default instruction must always be the "*stop_program ()*" function.

set_pump (rpm, direction, enable) controls the peristaltic pump. The function accepts values of byte-type (0–255). For the "*enable*" parameter, 0 corresponds to stop while any other positive integer corresponds to start. For the "*direction*" parameter, 0 corresponds to clockwise while any other positive integer corresponds to counterclockwise (the actual direction of the flow depends on the tube placement). The value of the "*rpm*" parameter controls the actual speed of the pump in RPM (revolutions per minute).

next_sample (N_of_samples, direction) controls the stepper motor of the autosampler. The function accepts values of "integer" type. For the "direction" parameter, 0 corresponds to clockwise while any other positive integer corresponds to counterclockwise. The autosampler rotates 1/*N_of_samples* of a full rotation.

set_temp_hum (target_T, target_H, enable) stores the setpoints for *T* and *H* and enables/disables the climate-control. The function accepts values of integer-type. For the "*enable*" parameter, 0 corresponds to stop, while any other positive integer corresponds to start.

reach_target_temp_hum () causes the sequence to stall until both temperature and humidity reach the setpoints within a range of ± 2 °C for *T* and ± 3% for *H*.

wait_milliseconds (x) causes the sequence to stall for × milliseconds before moving to the next instruction (ongoing active operations such as ambient control or pump rotation will keep being active). The function accepts values of unsigned_long-type.

instruction_loop (instr_number, number_of_cycles) is used to perform loops of several instructions. The program will jump back to the instruction indicated by "*instr_number*" for a number of times specified by "*number_of_cycles*".

stop_program () is the "default" instruction, meaning that it is performed any time at the end of the program routine. This function disables the automated program and restores all values to default.

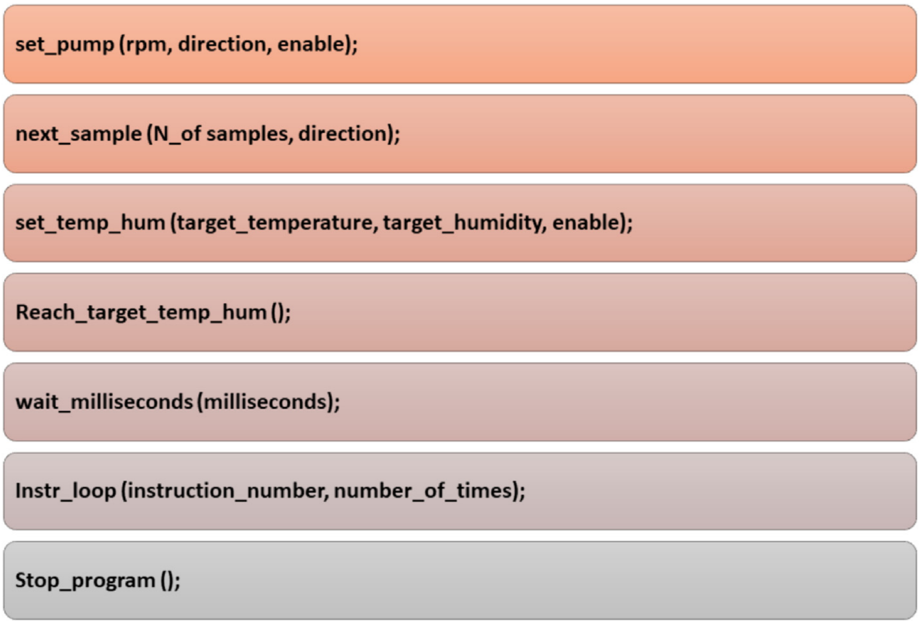


Fig. 21. Graphical overview of commands for customized automated programs.

6.4. Structure of Arduino sketch for pro Mini pump control

The Arduino Pro Mini has the only task to control the stepper motor of the peristaltic pump. It reads speed, direction and state values sent by the Arduino Mega 2560 from the serial bus and generates the digital signal for direction as well as the square wave signal required to control the stepper motor. This additional external microcontroller is required to provide accurate speed control of the pump, independently from the other functions which are handled by the main microcontroller (Arduino Mega 2560) to avoid critical timing issues. The code for the Arduino Pro Mini (*mini_pump_control.ino*) must be uploaded before connecting it to the circuit as it will not be accessible from the outside.

7. Cost analysis

The cost analysis for this project estimates a total cost of 494 USD for a complete retention model setup. As pricing of the various components can be heterogeneous depending on the source, the total cost is expected to vary depending on where the parts are purchased. However, we estimate that the cost is more likely to be reduced as in some cases parts were purchased that were more expensive than necessary. When looking at the cost analysis overview in the table, it becomes clear that the acrylic sheets as well as the electronics contribute the main expenses to the project.

Component groups		Cost (USD)
3D printed parts		45
Acrylic sheets		108
Electronics		246
Screws and other mechanical parts		88
Pump tubing		7
	Total Cost	**494**

The described retention model setup represents a highly customized solution and no commercial alternatives are available yet. Consequently, a cost comparison with commercial solutions is not possible. However, when comparing the cost of individual elements of the setup, such as the peristaltic pump or the temperature and humidity-controlled chamber, with their commercial counterparts, the presented solutions outcompete the latter by far. A comparable commercially available peristaltic pump (120S/DV, Watson-Marlow Danmark A/S, Denmark) that was purchased at a price of 1080 USD is more than twice as expensive as the entire retention model setup and does not offer the possibility of integration with an Arduino microcontroller. Similarly, a commercially available temperature and humidity-controlled chamber for laboratory purposes with approximately twice the volume (HCP 50, Memmert GmbH + Co. KG, Germany) could be found at a price of 7499 USD, thus being >15 times more expensive than the entire retention model setup [23]. These examples illustrate that besides being a customized solution with targeted functionality, the proposed retention model setup can drastically reduce laboratory expenses.

8. Validation and characterization

The functionality of the constructed retention model setup was tested. In this regard, we investigated the climate control over time, the performance of the peristaltic pump and the automation of the system.

8.1. Performance of climate control feedback loop

The capability of the climate control feedback loop using a ceramic heat lamp, an ultrasonic mist fogger, three fans, two DHT22 sensors and an Arduino Mega 2560 was tested by measuring temperature and relative humidity over time (Fig. 22).

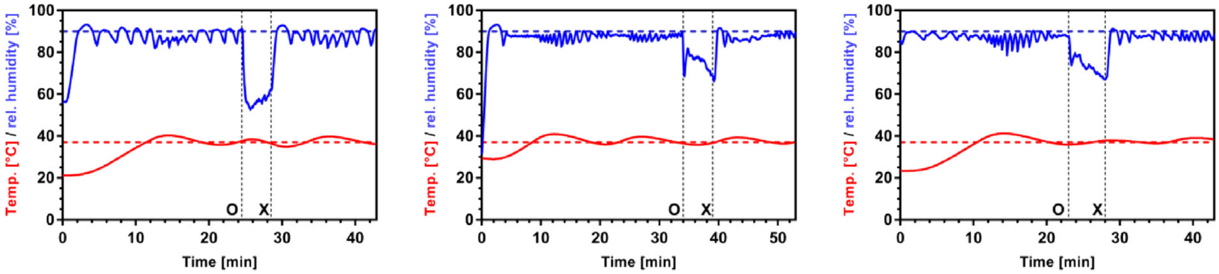


Fig. 22. Replicated measurements of temperature and relative humidity over time. Dotted lines represent the programmed target values. Events “O” and “X” mark when the door of the climate chamber was opened (“O”) and closed (“X”).

As experimentally relevant target values, we chose a temperature of 37 °C and a relative humidity of 90%. The results show that the humidity fluctuates right below the target value in a much faster frequency than temperature fluctuates around the target value. Furthermore, the target humidity can be reached much faster than the target temperature, which is reached after approximately 10 min. All in all, the control feedback loop shows a very robust behavior which can also be observed when the door of the chamber was opened for a specific amount of time. While the temperature stays unaffected after these short interruptions, the humidity decreases as an extreme down to 50% relative humidity but reaches the target value again within one minute after the event. The temperature exhibits a fluctuation in a range of 5.3 °C with an average of 37.9 °C and a standard deviation of ±1.5 °C. The fluctuation of relative humidity takes place in a range of 12% with an average of 88% and a standard deviation of ±2%.

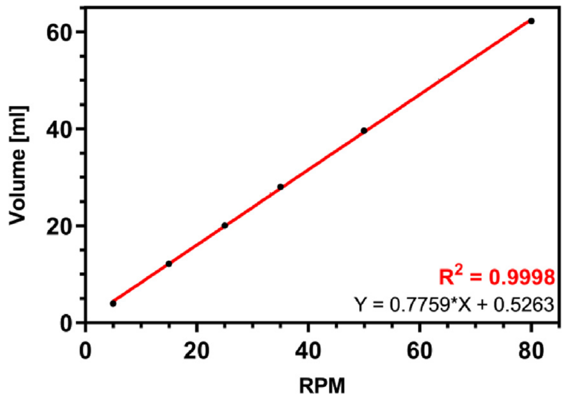


Fig. 23. Calibration curve of peristaltic pump. Error bars representing standard deviation are not depicted as they are smaller than the individual points of the plot. N = 3.

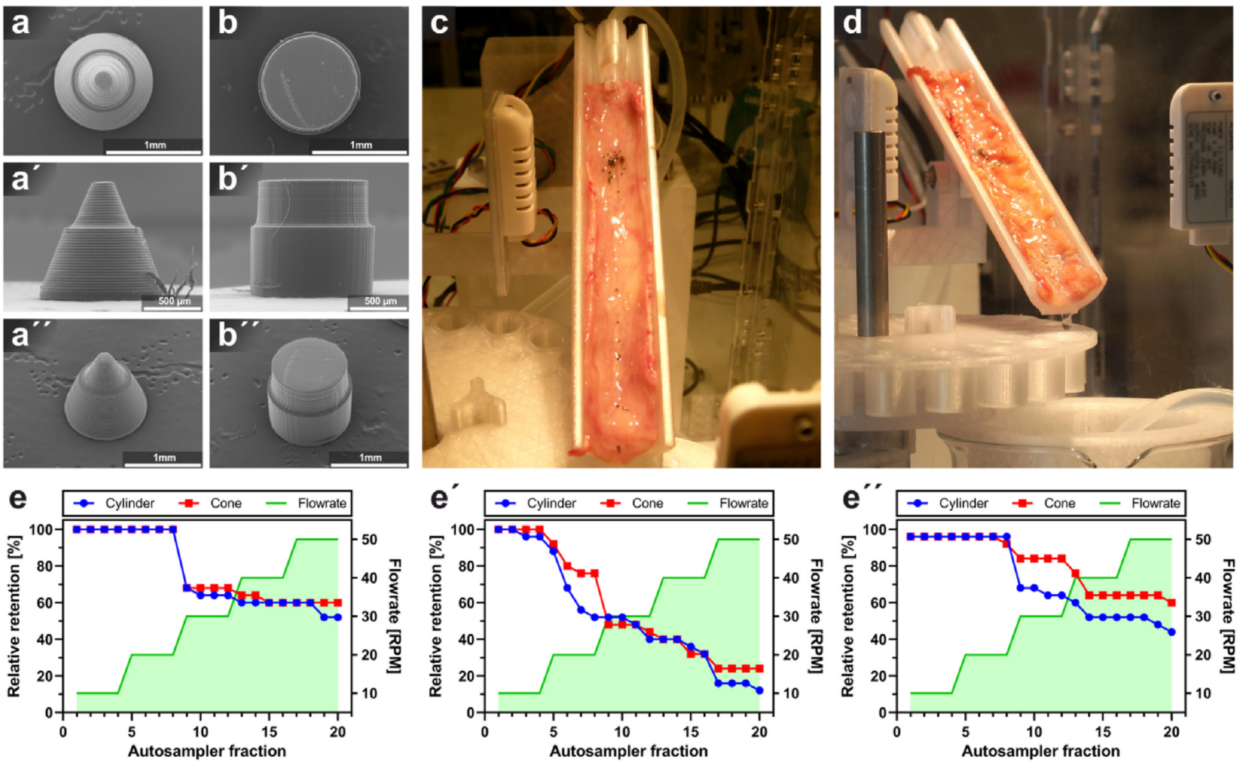


Fig. 24. Example retention measurement experiment using customized program automation. (a) Top view of millimeter sized 3D printed cone-shaped sample. (b) Top view of millimeter sized 3D printed cylinder-shaped sample. (‘) side view and (‘’) 45°tilted view in both cases. (c) Photograph of running experiment with both types of samples placed on the tissue. (d) Photograph of experimental setup with rotational autosampler and porcine intestinal tissue on 30°tilted tissue holder. (e) Graphical analysis of the data obtained from counting the samples remaining in the different fractions of the autosampler after three replicates (e – first replicate, e' – second replicate, e'' – third replicate).

8.2. Peristaltic pump calibration

The peristaltic pump was adapted from a previously posted project in which it was characterized in detail [24]. As the pump was introduced to a modified setting and modified control, a calibration curve was determined (Fig. 23). Within the examined RPM-range, the pump exhibits a precise linear relationship to the flowrate.

8.3. Demonstration of retention experiment using automated program sequence and autosampler

To demonstrate the extended capabilities by means of automation of the presented retention model setup, a demonstration experiment with two differently shaped 3D printed specimens was performed (Fig. 24). A piece of untreated porcine intestinal tissue was placed on the tissue holder as soon as the climate control reached the target values of 37 °C and 90% relative humidity. Then the tissue was flushed with Dulbeccos' phosphate buffered saline for 5 min at 50 RPM to remove loose pieces of mucus. The 3D printed cones and cylinders (Fig. 24a and b) had a diameter of 1 mm and were placed simultaneously and randomly in a number of 25 each onto the intestine and left for incubation for 10 min (Fig. 24c). With expiration of the incubation time, a customized program as visualized in Table 1 was started.

As indicated, the program started the pump at 10 RPM, then rotated the autosampler to the next sample (small filter mesh; Fig. 24d) four times after two minutes of flow each. Then the flow was increased consecutively three times to 30, 40 and 50 RPM in order to increase stress conditions. After the program stopped, the cone and cylinder samples in the fractions of the autosampler as well as the samples remaining on the tissue were counted. Plotting the results of the replicated experiments as relative amount of samples left on the tissue (relative retention) against the number of autosampler fractions (Fig. 24e) shows that in all replicates there is a tendency of the cones to exhibit slightly higher retention than the cylinders. This could be caused by the fact that the cones encounter less drag force than the cylinders and also as they might penetrate

Table 1
Example instruction loop used for retention experiment in Fig. 24. Climate control is set to a temperature of 37 °C and a relative humidity of 90%. After a waiting time of 5 s, the flow of the pump is started at a flowrate of 10 RPM. After 2 min, the sample in the autosampler is changed. The latter procedure is repeated 4 times, then the flowrate is increased. The flow rate is increased 4 times in total until the program stops.

```
void my_program()
{

switch (k) {

 case 0:
 set_temp_hum(37,90,1);
 break;
 case 1:
 wait_milliseconds(5000);
 break;

 case 2:
 set_pump(10,1,1);
 break;
 case 3:
 wait_milliseconds(120000);
 break;
 case 4:
 next_sample(20,0);
 break;
 case 5:
 instruction_loop(3, 3);
 break;
 case 6:
 set_pump(20,1,1);
 break;
 case 7:
 wait_milliseconds(120000);
 break;
 case 8:
 next_sample(20,0);
 break;
 case 9:
 instruction_loop(7, 3);
 break;

 case 10:
 set_pump(30,1,1);
 break;
 case 11:
 wait_milliseconds(120000);
 break;
 case 12:
 next_sample(20,0);
 break;
 case 13:
 instruction_loop(11, 3);
 break;
 case 14:
 set_pump(40,1,1);
 break;
 case 15:
 wait_milliseconds(120000);
 break;
 case 16:
 next_sample(20,0);
 break;
 case 17:
 instruction_loop(15, 3);
 break;

 case 18:
 set_pump(50,1,1);
 break;
 case 19:
 wait_milliseconds(120000);
 break;
 case 20:
 next_sample(20,0);
 break;
 case 21:
 instruction_loop(19, 3);
 break;
 default:
 stop_program();
 break;

 }

}
```

into the mucus layer more easily due to their sharp tip. Since the experiment was fully automated, the number of autosampler fraction correlates with time as well as with flowrate.

In summary, the demonstration and validation of the instrument shows:

- Stable control of temperature and humidity over time
  - o Fluctuation of temperature in range of 5.3 °C over time (mean of 37.9 °C; standard deviation of ±1.5 °C over time)
  - o Fluctuation of relative humidity in range of ~12% over time (mean of 88%; standard deviation of ± 2% over time)
- Precise peristaltic pump calibration with linear correlation of RPM to flowrate ml/min in a physiologically relevant range of flowrate
- Capability to run fully automated mucoadhesion assays using an autosampler and a customized controller program

9. Potential modifications

Potential modifications of the system include a more precise control of the slide angle. Increased repeatability of the angle will possibly lead to increased repeatability of the experiments. As the RAMPS 1.4 Arduino shield has three more slots for stepper motor drivers, a motor could be used to precisely control the angle of the slide.

Further, the design of the peristaltic pump could be altered in order to more easily fit tubes of different diameters and thereby achieve different ranges of flowrates.

Another desirable modification would be the optimization of 3D printed parts to reduce the amount of required screws and to adapt the parts to the use of the same size and type of screws as they contribute a substantial fraction of the cost of the setup.

Declaration of Competing Interest

None.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ohx.2019.e00071.

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